HIV-1 VIRION MATURATION INHIBITORS, COMPOSITIONS AND TREATMENTS USING THE SAME

Cross Reference To Related Application

This application claims the benefit of U. S. Provisional Application Serial No. 60/663,049, filed March 17, 2005, the contents of which is hereby incorporated by reference in it's entirety.

Field of the Invention

The present invention relates to compounds that are useful as inhibitors of HIV-1 antiviral activity, pharmaceutical compositions comprising such compounds, methods of using such compounds and formulations in the treatment of HIV-infected mammals, such as humans, and methods and intermediate compounds useful in preparing such compounds. These anti-HIV compounds may be used alone or in combination with other antiviral compounds. More specifically, the invention relates to diphenylacetamide derivatives that block HIV-1 replication by directly or indirectly interfering with the processing of the HIV-1 gp160 envelope precursor into the mature functional envelope proteins gp120 and gp41. These compounds exploit a new mechanism in the HIV-1 replication cycle that has not been targeted previously with small molecule inhibitors.

Background of the Invention

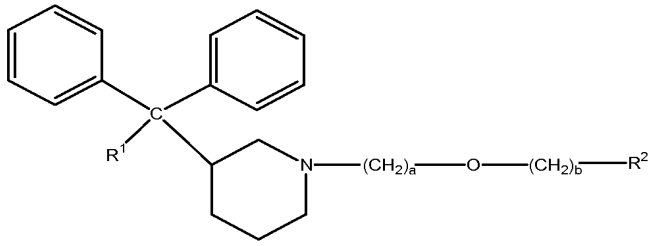
HIV remains a global health problem with estimated 38 million people infected worldwide (UNAIDS) and an increasing infection rate in many countries, particularly in Asia (Cohen, 2004). Presently there are 24 drugs or combinations of drugs approved for the treatment of HIV-1 infection that fall into one of four therapeutic classes: the nucleoside reverse transcriptase inhibitors (NRTIs), the non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), and fusion inhibitors. Although clinically effective when used in combination, none of the currently available drugs represent ideal therapies, due to drug-related side effects, inconvenient dosing requirements, and/or the emergence of drug resistant virus (reviewed in Yeni et al., 2002). The issue of drug resistance is particularly problematic given that viral variants resistant to one drug of a particular class often exhibit some level of cross-resistance to other dugs within the same class. Therefore, there still exists a critical need for the identification and development of new HIV-1 inhibitors that exhibit improved safety profiles and that are effective against HIV-1 variants resistant to the current drug classes.

The identification of HIV-1 inhibitors that act against novel targets represents one approach toward addressing the issue of HIV-1 drug resistance. Antiviral compounds that target new mechanisms, including HIV-1 integrase (IN) inhibitors (Billich, 2003; Pais and Burke, 2002), a CCR5 inhibitor (Reynes et al., 2002), a gp120/CD4 inhibitor (Hanna et al.

2004), and a virion maturation inhibitor (Wild et al., 2003), are currently in early clinical development. It is expected that these compounds will be effective against HIV-1 variants resistant to the currently marketed classes of drugs. Although these compounds appear promising, the long term clinical utility of these agents remains to be demonstrated. Previously, peptide-based inhibitors have been described that inhibit the activity of the cellular protease(s) that mediate HIV-1 envelope processing (Angliker, 1995, J. Med. Chem. 38: 4014-4018; Bahbouhi, et al., 2001, Biochem. J. 360: 127-134; Garten, et al., 1994, Biochemie 76:217-225; Hallenberger et al., 1992, Nature 360:358-361). However, the peptide-like structure of these inhibitors limit their ability to penetrate cells and thus limit their therapeutic potential. In addition, the function of furin and related proteases mediate important processes in cells and inhibition of such proteases may result in toxicity.

SUMMARY OF THE INVENTION

The present invention relates to compounds of Formula I:

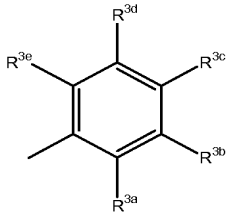


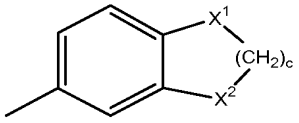
I

wherein:

R^1 is –CN or $–CONR^4R^5$;

R^2 is C_1-C_4 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 heterocycloalkyl, C_6-C_{14} aryl, or a group of the formula:





or Het;

R^{3a}, R^{3b}, R^{3c}, R^{3d} and R^{3e} are each independently H, C_1-C_4 alkyl, C_1-C_4 alkoxy, $–(CH_2)_dOH$, halo, trifluoromethyl, cyano, $–(CH_2)_dNR^6R^7$, $–CO(C_1$-C_4 alkyl), $–OCO(C_1$-C_4 alkyl), $–CH(OH)(C_1$-C_4 alkyl), $–C(OH)(C_1$-C_4 alkyl$)_2$, $–SO_2NH_2$, $–(CH_2)_dCONR^8R^9$ or $–(CH_2)_dCOO(C_1$-C_4 alkyl);

R^4, R^5, R^6, R^7, R^8 and R^9 are each independently H or C_1-C_4 alkyl;

Het is pyridyl, pyrazinyl or thienyl;

a is 1, 2, 3 or 4;

b is 1, 2 or 3;

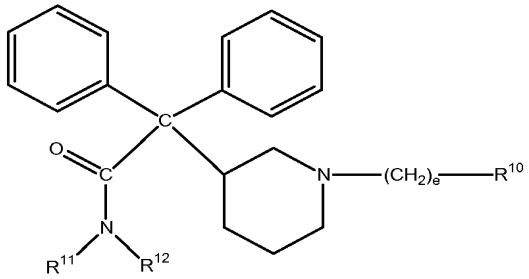
c is 1, 2 or 3;

d is 0, 1 or 2; and

X^1 and X^2 are each independently CH_2 or O;

or pharmaceutically acceptable salts or solvates thereof.

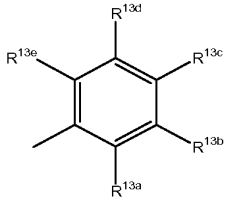
The present invention further relates to compounds of Formula II:

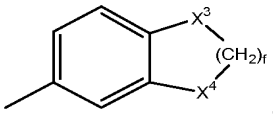


II

wherein:

R^{10} is a group of the formula:



,  , or Het;

R^{11} and R^{12} are each independently H or C_1-C_4 alkyl, with the proviso that R^{11} and R^{12} are not both H;

R^{13a}, R^{13b}, R^{13c}, R^{13d}, and R^{13e} are each independently H, C_1-C_4 alkyl, C_1-C_4 alkoxy, $-(CH_2)_gOH$, halo, trifluoromethyl, cyano, $-(CH_2)_gNR^{14}R^{15}$, $-CO(C_1$-C_4 alkyl), $-OCO(C_1$-C_4 alkyl), $-CH(OH)(C_1$-C_4 alkyl), $-C(OH)(C_1$-C_4 alkyl$)_2$, $-SO_2NH_2$, $-(CH_2)_gCONR^{16}R^{17}$ or $-(CH_2)_gCOO(C_1$-C_4 alkyl);

R^{14}, R^{15}, R^{16} and R^{17} are each independently H or C_1-C_4 alkyl;

Het is pyridyl, pyrazinyl or thienyl;

e is 1, 2 or 3;

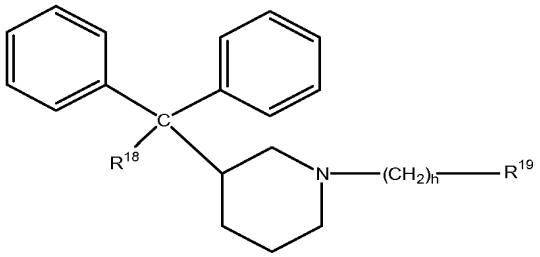
f is 1, 2 or 3;

g is 0, 1 or 2; and

X^3 and X^4 are each independently CH_2 or O;

or pharmaceutically acceptable salts or solvates thereof.

The present invention further relates to compounds of Formula III:



III

wherein:

R^{18} is –CN or $–CONR^{20}R^{21}$;

R^{19} is C_3-C_6 cycloalkyl, C_3-C_6 heterocycloalkyl or (C_6-C_{14} aryl)–(C_1-C_4 alkyl)$_v$;

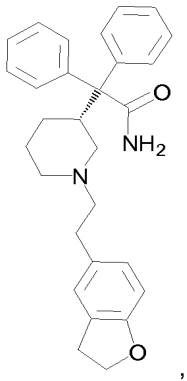
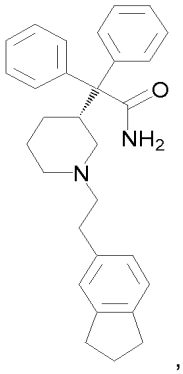
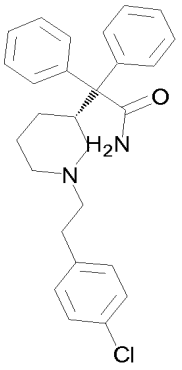
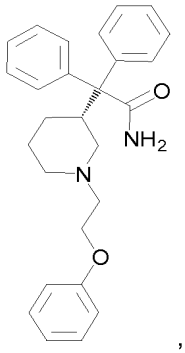
R^{20} and R^{21} are each independently H or C_1-C_4 alkyl;

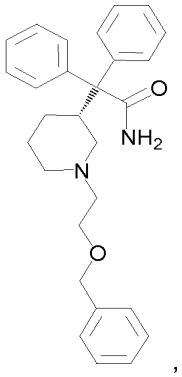
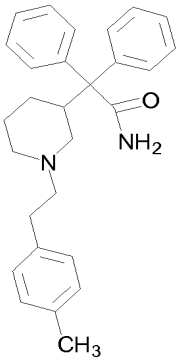
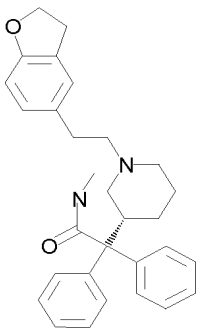
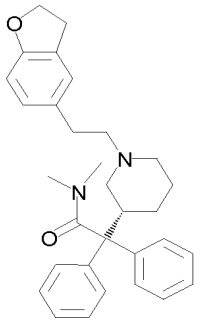
h is 1, 2, 3 or 4; and

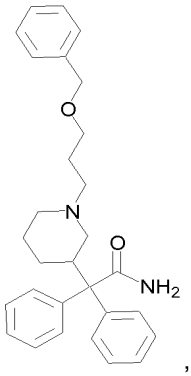
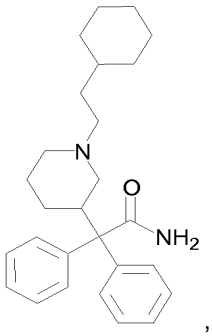
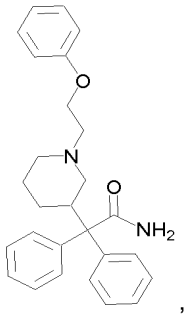
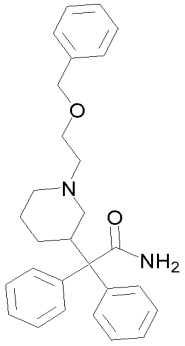
v is 0, 1 or 2;

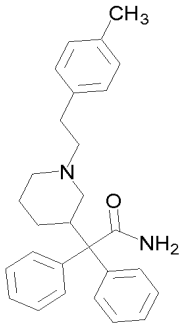
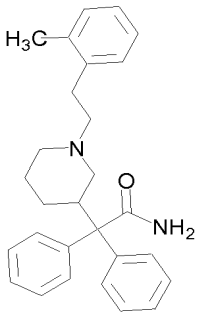
or pharmaceutically acceptable salts or solvates thereof.

In yet another aspect are compounds selected from:


,

,

,

,


,

,

,

,

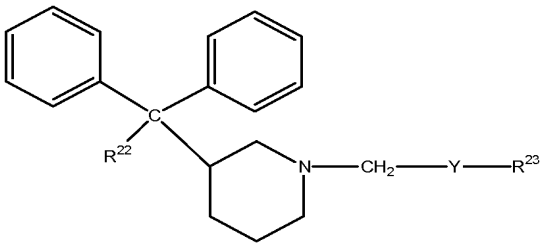

,

,

,

,


and

,

or pharmaceutically acceptable salts or solvates thereof.

The present invention also relates to a method of treating a mammal infected with human immunodeficiency virus (HIV) comprising administering to said mammal an effective amount of a compound of Formula I, II or III.

The present invention further relates to a method of treating a mammal infected with human immunodeficiency virus (HIV) comprising administering to said mammal an effective amount of a compound according to Formula IV:



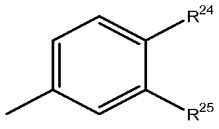
IV

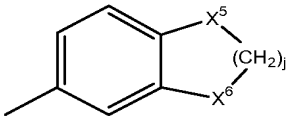
wherein:

Y is a direct link, $–CH_2–$, $–(CH_2)_2–$, $–CH_2O–$ or $–CH_2S–$;

R^{22} is $–CN$ or $–CONH_2$;

R^{23} is a group of the formula:



,


or Het;

wherein

R^{24} and R^{25} are each independently H, C_1-C_4 alkyl, C_1-C_4 alkoxy, $-(CH_2)_kOH$, halo, trifluoromethyl, cyano, $-(CH_2)_kNR^{26}R^{27}$, $-CO(C_1$-C_4 alkyl), $-OCO(C_1$-C_4 alkyl), $-CH(OH)(C_1$-C_4 alkyl), $-C(OH)(C_1$-C_4 alkyl$)_2$, $-SO_2NH_2$, $-(CH_2)_kCONR^{26}R^{27}$ or $-(CH_2)_kCOO(C_1$-C_4 alkyl);

R^{26} and R^{27} are each independently H or C_1-C_4 alkyl;

k is 0, 1 or 2;

X^5 and X^6 are each independently O or CH_2;

j is 1, 2 or 3; and

Het is pyridyl, pyrazinyl or thienyl;

or a pharmaceutically acceptable salt or solvate thereof.

The present invention further relates to a method of inhibiting HIV activity in a mammal infected with HIV comprising administering to said mammal an effective amount of compounds of Formula I, II, III or IV.

The present invention also relates to a method of inhibiting HIV replication in a mammal infected with HIV comprising administering to said mammal an effective amount of a compound of Formula I, II, III or IV.

The present invention further relates to a pharmaceutical composition comprising an effective amount of a compound of Formula I, II, III or IV that is effective in treating HIV in an infected mammal, and a pharmaceutically acceptable carrier.

The present invention also relates to a use of a compound of Formula I, II, III or IV in the preparation of a medicament for the treatment of a mammal suffering from infection with HIV.

The present invention further relates to a pharmaceutical composition that is effective in treating HIV in an infected mammal comprising a pharmaceutically acceptable carrier and an effective amount of a compound of Formula I, II, III or IV.

The present invention also relates to a pharmaceutical composition that is effective in treating HIV in an infected mammal comprising a pharmaceutically acceptable carrier and an effective amount of a compound selected from 1–(4–chlorophenethyl)–3–(R)–(+)–(1–cyano–1,1–diphenylmethyl)piperidine; 3–(R)–(+)–(1–cyano–1,1–diphenylmethyl)–1–phenethylpiperidine; 3–(R)–(+)–(1–cyano–1,1–diphenylmethyl)–1–[2–(2,3–dihydrobenzofuran–5–yl)ethyl]piperidine; 3–(R)–(+)–(1–cyano–1,1–diphenylmethyl)–1–(4–hydroxymethylphenethyl)piperidine; 3–(R)–(1–cyano–1,1–diphenylmethyl)–1–[2–indan–5–yl)ethyl]piperidine; 3–(R)–(1–cyano–1,1–diphenylmethyl)–1–(3–methyl–phenethyl)piperidine; 3–(R)–(+)–(1–cyano–1,1–diphenylmethyl)–1–(4–methylphenethyl)piperidine; 3–(R)–(1–

cyano–1,1–diphenylmethyl)–1–(3,4–dichlorophenethyl)piperidine; 3–(R,S)–(1–cyano–1,1–diphenylmethyl)–1–(3–phenyl–propyl)piperidine; 3–(R,S)–(1–cyano–1,1–diphenylmethyl)–1–(4–hydroxyphenethyl)piperidine; 3–(R,S)–(1–cyano–1,1–diphenylmethyl)–1–(4–carbamoylphenethyl)piperidine; 3–(R,S)–(1–cyano–1,1–diphenylmethyl)–1–(4–methoxyphenethyl)piperidine; 3–(R,S)–(1–cyano–1,1–diphenylmethyl)–1–(3,4–methylenedioxybenzyl)piperidine; 3–(R)–(1–cyano–1,1–diphenylmethyl)–1–(2–phenoxyethyl)piperidine; 3–(R)–(1–cyano–1,1–diphenylmethyl)–1–(4–hydroxy–3–methoxyphenethyl)piperidine; 3–(R,S)–(1–carbamoyl–1,1–diphenylmethyl)–1–(3,4–methylenedioxybenzyl)piperidine; 3–(R,S)–(1–carbamoyl–1,1–diphenylmethyl)–1–(4–methoxyphenethyl)piperidine; 3–(R,S)–(1–carbamoyl–1,1–diphenylmethyl)–1–[3–(4–methoxyphenyl)propyl]piperidine; 3–(R,S)–(1–carbamoyl–1,1–diphenylmethyl)–1–(4–methylphenethyl)piperidine; 3–(R,S)–(1–carbamoyl–1,1–diphenylmethyl)–1–phenethylpiperidine; 3–(R,S)–(1–carbamoyl–1,1–diphenylmethyl)–1–(4–carbamoylphenethyl)piperidine; 3–(R,S)–(1–carbamoyl–1,1–diphenylmethyl)–1–(4–chlorophenethyl)piperidine; and 3–(R,S)–(1–carbamoyl–1,1–diphenylmethyl)–1–(4–hydroxyphenethyl)piperidine.

The present invention further relates to a composition comprising a compound according to Formula I, II, III or IV, or any of the preceding named compounds, and at least one additional therapeutic agent chosen from nucleoside HIV reverse transcriptase inhibitors, non-nucleoside HIV reverse transcriptase inhibitors, HIV protease inhibitors, HIV integrase inhibitors, HIV fusion inhibitors, immune modulators, CCR5 antagonists, and antiinfectives.

The present invention also relates to a composition described above wherein said additional therapeutic agent is chosen from nelfinavir, ritonavir, lopinavir, kaletra, efavirenz, nevirapine, lamivudine, zidovudine, and tenofovir.

The present invention further relates to a method for treating an HIV infection in an infected mammal comprising administering a compound according to Formula I, II, III or IV, or any of the preceding compounds, and at least one additional therapeutic agent chosen from nucleoside HIV reverse transcriptase inhibitors, non-nucleoside HIV reverse transcriptase inhibitors, HIV protease inhibitors, HIV integrase inhibitors, HIV fusion inhibitors, immune modulators, CCR5 antagonists, and antiinfectives.

The present invention also relates to the use of a compound according to Formula I, II, III or IV, or any of the preceding compounds, and at least one additional therapeutic agent chosen from nucleoside HIV reverse transcriptase inhibitors, non-nucleoside HIV reverse transcriptase inhibitors, HIV protease inhibitors, HIV integrase inhibitors, HIV fusion inhibitors, immune modulators, CC5 antagonists, and antiinfectives, in the manufacture of a medicament for the treatment of HIV infection in a mammal in need of such treatment.

As used herein, the terms “comprising” and “including” are used in their open, non-limiting sense.

The term "C_1-C_4 alkyl", as used herein, unless otherwise indicated, includes saturated monovalent hydrocarbon radicals having straight, branched, or cyclic moieties (including fused and bridged bicyclic and spirocyclic moieties), or a combination of the foregoing moieties, and containing from 1-4 carbon atoms. For an alkyl group to have cyclic moieties, the group must have at least three carbon atoms.

A “lower alkyl” is intended to mean an alkyl group having from 1 to 4 carbon atoms in its chain. The term “heteroalkyl” refers to a straight- or branched-chain alkyl group having from 2 to 12 atoms in the chain, one or more of which is a heteroatom selected from S, O, and N. Exemplary heteroalkyls include alkyl ethers, secondary and tertiary amines, alkyl sulfides and the like.

The term "C_2-C_6 alkenyl", as used herein, unless otherwise indicated, includes alkyl moieties having at least one carbon-carbon double bond wherein alkyl is as defined above and including E and Z isomers of said alkenyl moiety, and having from 2 to 6 carbon atoms.

The term "C_2-C_6 alkynyl", as used herein, unless otherwise indicated, includes alkyl moieties having at least one carbon-carbon triple bond wherein alkyl is as defined above, and containing from 2-6 carbon atoms.

The term “carbocycle” refers to a saturated, partially saturated, unsaturated, or aromatic, monocyclic or fused or non-fused polycyclic, ring structure having only carbon ring atoms (no heteroatoms, i.e., non-carbon ring atoms). Exemplary carbocycles include cycloalkyl, aryl, and cycloalkyl-aryl groups.

A "C_3-C_{10} cycloalkyl group" is intended to mean a saturated or partially saturated, monocyclic, or fused or spiro polycyclic, ring structure having a total of from 3 to 10 carbon ring atoms (but no heteroatoms). Exemplary cycloalkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cycloheptyl, adamantyl, and like groups.

A "heterocycloalkyl group" is intended to mean a monocyclic, or fused or spiro polycyclic, ring structure that is saturated or partially saturated, and has a total of from 3 to 18 ring atoms, including 1 to 5 heteroatoms selected from nitrogen, oxygen, and sulfur. Illustrative Examples of heterocycloalkyl groups include pyrrolidinyl, tetrahydrofuryl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, aziridinyl, and like groups.

The term "C_6-C_{10} aryl", as used herein, unless otherwise indicated, includes an organic radical derived from an aromatic hydrocarbon by removal of one hydrogen, such as phenyl or naphthyl. The term “phenyl” and the symbol “Ph,” as used herein, refer to a C_6H_5 group.

The term "4-10 membered heterocyclic", as used herein, unless otherwise indicated, includes aromatic and non-aromatic heterocyclic groups containing one to four heteroatoms each selected from O, S and N, wherein each heterocyclic group has from 4-10 atoms in its ring system, and with the proviso that the ring of said group does not contain two adjacent O or S atoms. Furthermore, the sulfur atoms contained in such heterocyclic groups may be oxidized with one or two sulfur atoms. Non-aromatic heterocyclic groups include groups having only 4 atoms in their ring system, but aromatic heterocyclic groups must have at least 5 atoms in their

ring system. The heterocyclic groups include benzo-fused ring systems. An example of a 4 membered heterocyclic group is azetidinyl (derived from azetidine). An example of a 5 membered heterocyclic group is thiazolyl and an example of a 10 membered heterocyclic group is quinolinyl. Examples of non-aromatic heterocyclic groups are pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, piperidino, morpholino, thiomorpholino, thioxanyl, piperazinyl, azetidinyl, oxetanyl, thietanyl, homopiperidinyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 1,2,3,6-tetrahydropyridinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, 3-azabicyclo[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, 3H-indolyl and quinolizinyl. Examples of aromatic heterocyclic groups are pyridinyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxazolyl, oxadiazolyl, isoxazolyl, isothiazolyl, pyrrolyl, quinolinyl, isoquinolinyl, indolyl, benzimidazolyl, benzofuranyl, cinnolinyl, indazolyl, indolizinyl, phthalazinyl, pyridazinyl, triazinyl, isoindolyl, pteridinyl, purinyl, oxadiazolyl, thiadiazolyl, furazanyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl, and furopyridinyl. The foregoing groups, as derived from the groups listed above, may be C-attached or N-attached where such is possible. For instance, a group derived from pyrrole may be pyrrol-1-yl (N-attached) or pyrrol-3-yl (C-attached). Further, a group derived from imidazole may be imidazol-1-yl (N-attached) or imidazol-3-yl (C-attached). An example of a heterocyclic group wherein 2 ring carbon atoms are substituted with oxo (=O) moieties is 1,1-dioxo-thiomorpholinyl.

The term “5-6 membered heterocyclic” means aromatic and non-aromatic heterocyclic groups containing one to four heteroatoms each selected from O, S and N, and wherein each heterocyclic group has a total of from 5 to 6 atoms in its ring system, and with the proviso that the ring of said group does not contain two adjacent O or S atoms. The sulfur atoms contained in such heterocyclic groups may be oxidized with one or two sulfur atoms. Furthermore, any atom in the 5-6 membered heterocyclic group may be substituted with an oxo (=O) group, if such substitution would result in a stable compound. Examples of non-aromatic heterocyclic groups include, but are not limited to, pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, piperidino, morpholino, thiomorpholino, thioxanyl, piperazinyl, azetidinyl, oxetanyl, thietanyl, homopiperidinyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 1,2,3,6-tetrahydropyridinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, 3-azabicyclo[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, 3H-indolyl and quinolizinyl. Examples of aromatic heterocyclic groups include, but are not limited to, pyridinyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxazolyl, isothiazolyl, pyrrolyl,

quinolinyl, isoquinolinyl, indolyl, benzimidazolyl, benzofuranyl, cinnolinyl, indazolyl, indolizinyl, phthalazinyl, pyridazinyl, triazinyl, isoindolyl, pteridinyl, purinyl, oxadiazolyl, thiadiazolyl, furazanyl, quinazolinyl, and quinoxalinyl. The foregoing groups, as derived from the groups listed above, may be C-attached or N-attached where such is possible. For instance, a group derived from pyrrole may be pyrrol-1-yl (N-attached) or pyrrol-3-yl (C-attached). Further, a group derived from imidazole may be imidazol-1-yl (N-attached) or imidazol-3-yl (C-attached). An example of a heterocyclic group wherein 2 ring carbon atoms are substituted with oxo (=O) moieties is 1,1-dioxo-thiomorpholinyl.

A "heteroaryl group" is intended to mean a monocyclic or fused or spiro polycyclic, aromatic ring structure having from 4 to 18 ring atoms, including from 1 to 5 heteroatoms selected from nitrogen, oxygen, and sulfur. Illustrative Examples of heteroaryl groups include pyrrolyl, thienyl, oxazolyl, isoxazolyl, pyrazolyl, thiazolyl, furyl, pyridinyl, pyrazinyl, triazolyl, tetrazolyl, indolyl, quinolinyl, quinoxalinyl, benzthiazolyl, benzodioxinyl, benzodioxolyl, benzooxazolyl, oxadiazolyl, and the like.

The term "alkoxy", as used herein, unless otherwise indicated, includes O-alkyl groups wherein alkyl is as defined above.

The term “amino” is intended to mean the $–NH_2$ radical.

The terms “halogen” and “halo,” as used herein represent fluorine, chlorine, bromine or iodine.

The term “oxo,” as used herein, means a group (=O). Such a group may be bonded to either a carbon atom or a heteroatom in the compounds of the present invention, if such substitution will result in a stable compound.

The term “trifluoromethyl,” as used herein, is meant to represent a group $–CF_3$.

The term “trifluoromethoxy,” as used herein, is meant to represent a group $–OCF_3$.

The term “cyano,” as used herein, is meant to represent a group –CN.

The term “substituted” means that the specified group or moiety bears one or more substituents. The term “unsubstituted” means that the specified group bears no substituents. The term “optionally substituted” means that the specified group is unsubstituted or substituted by one or more substituents.

The term “HIV,” as used herein, refers to human immunodeficiency virus.

The terms “inhibiting HIV” and “inhibiting HIV replication” mean inhibiting HIV replication either *in vitro* or *in vivo*, such as in a mammal, such as a human, by contacting the virus with an HIV-replication inhibiting amount of a compound of the present invention, or a pharmaceutically acceptable salt or solvate thereof. Such inhibition may take place *in vivo*, such as in a mammal, such as a human, by administering to the mammal an HIV-inhibiting amount of a compound of the present invention. The amount of a compound of the present invention necessary to inhibit replication of the HIV virus either *in vitro* or *in vivo*, such as in a mammal, such as a human, can be determined using methods known to those of ordinary skill in the art. For example, an amount of a compound of the invention may be administered

to a mammal, either alone or as part of a pharmaceutically acceptable formulation. Blood samples may then be withdrawn from the mammal and the amount of HIV virus in the sample may be quantified using methods known to those of ordinary skill in the art. A reduction in the amount of HIV virus in the sample compared to the amount found in the blood before administration of a compound of the invention would represent inhibition of the replication of HIV virus in the mammal. The administration of a compound of the invention to the mammal may be in the form of single dose or a series of doses over successive days.

An “HIV-inhibiting agent” means a compound of the present invention or a pharmaceutically acceptable salt or solvate thereof.

The term “HIV-inhibiting amount,” as used herein, refers to an amount of a compound of the present invention that is sufficient to inhibit the replication of the human immunodeficiency virus when administered to a mammal, such as a human.

A "solvate" is intended to mean a pharmaceutically acceptable solvate form of a specified compound that retains the biological effectiveness of such compound. Examples of solvates include compounds of the invention in combination with solvents such as, but not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, or ethanolamine. A "pharmaceutically acceptable salt" is intended to mean a salt that retains the biological effectiveness of the free acids and bases of the specified derivative and that is not biologically or otherwise undesirable. Examples of pharmaceutically acceptable salts include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogenphosphates, dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrates, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, sulfonates, xylenesulfonates, phenylacetates, phenylpropionates, phenylbutyrates, citrates, lactates, γ-hydroxybutyrates, glycollates, tartrates, methane-sulfonates, propanesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, and mandelates.

The term "treating", as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term "treatment", as used herein, unless otherwise indicated, refers to the act of treating as "treating" is defined immediately above.

The phrase "pharmaceutically acceptable salt(s)", as used herein, unless otherwise indicated, includes salts of acidic or basic groups, which may be present in the compounds of the present invention. The compounds of the present invention that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds of the present invention are those that form non-toxic acid addition salts, i.e., salts

containing pharmacologically acceptable anions, such as the acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edislyate, estolate, esylate, ethylsuccinate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, phospate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodode, and valerate salts.

The phrases “therapeutically effective amount,” “effective amount,” and “HIV-inhibiting amount,” are intended to mean the amount of an inventive agent that, when administered to a mammal in need of treatment, is sufficient to effect treatment for injury or disease conditions alleviated by the inhibition of HIV replication such as for potentiation of anti-cancer therapies or inhibition of neurotoxicity consequent to stroke, head trauma, and neurodegenerative diseases. The amount of a given HIV-inhibiting agent used in the method of the invention that will be therapeutically effective will vary depending upon factors such as the particular HIV-inhibiting agent, the disease condition and the severity thereof, the identity and characteristics of the mammal in need thereof, which amount may be routinely determined by artisans.

As used herein, the term “catalyst” means a chemical element or compound that increases the rate of a chemical reaction by reducing the activation energy, but which is left unchanged by the reaction. Examples of catalysts include, but are not limited to, palladium (0) and platinum (0). It is specifically contemplated herein that such catalysts may be formed *in situ* during the course of a chemical reaction, from a so-called “pre-catalyst,” but may never actually be observed or isolated. Such pre-catalysts are chemical compounds that are capable of being converted *in situ* during the course of a chemical reaction to a chemically and catalytically competent element or compound. Examples of suitable pre-catalysts include, but are not limited to, $PdCl_2$, $PdCl_2(PPh_3)_2$, $Pd(OH)_2$, $Pd(PPh_3)_4$, $Pt(OH)_2$, and $PtCl_2$.

The term “reducing agent,” as used herein, means a chemical element or compound that provides electrons for another chemical element or compound in a reaction mixture. Alternatively, it means a chemical element or compound that is capable of affording a saturated chemical compound from an unsaturated chemical compound by the addition of hydrogen. For example, the addition of hydrogen to an alkene of the present invention to afford a saturated alkane is termed “reduction.” A reducing agent is a chemical element or compound that is capable of affecting such a reduction, usually in the presence of a catalyst. Examples of reducing agents include, but are not limited to hydrogen, formic acid, and formic acid salts, such as ammonium formate.

The term “protecting,” as used herein, refers to a process in which a functional group in a chemical compound is selectively masked by a non-reactive functional group in order to allow a selective reaction(s) to occur elsewhere on said chemical compound. Such non-

reactive functional groups are herein termed "protecting groups." For example, the term "hydroxyl protecting group," as used herein refers to those groups that are capable of selectively masking the reactivity of a hydroxyl (-OH) group. The term "suitable protecting group," as used herein refers to those protecting groups that are useful in the preparation of the compounds of the present invention. Such groups are generally able to be selectively introduced and removed using mild reaction conditions that do not interfere with other portions of the subject compounds. Protecting groups that are suitable for use in the processes and methods of the present invention are known to those of ordinary skill in the art. The chemical properties of such protecting groups, methods for their introduction, and their removal can be found, for example, in T. Greene and P. Wuts, *Protective Groups in Organic Synthesis* (3rd ed.), John Wiley & Sons, NY (1999). The terms "deprotecting," "deprotected," or "deprotect," as used herein, are meant to refer to the process of removing a protecting group from a compound.

The terms "hydrolyze," "hydrolyzing," "hydrolysis," and "hydrolyzed," as used herein, all mean and refer to a chemical reaction in which an ester, an amide, or both are converted into their corresponding carboxylic acid derivatives, usually through the action of hydroxyl anion (-OH), such as would be present in a basic, aqueous solution.

The term "leaving group," as used herein, refers to a chemical functional group that generally allows a nucleophilic substitution reaction to take place at the atom to which it is attached. For example, in acid chlorides of the formula Cl-C(O)R, wherein R is alkyl, aryl, or heterocyclic, the –Cl group is generally referred to as a leaving group because it allows nucleophilic substitution reactions to take place at the carbonyl carbon to which it is attached. Suitable leaving groups are known to those of ordinary skill in the art and can include halides, aromatic heterocycles, cyano, amino groups (generally under acidic conditions), ammonium groups, alkoxide groups, carbonate groups, formates, and hydroxy groups that have been activated by reaction with compounds such as carbodiimides. For example, suitable leaving groups can include, but are not limited to, chloride, bromide, iodide, cyano, imidazole, and hydroxy groups that have been allowed to react with a carbodiimide such as dicyclohexylcarbodiimide (optionally in the presence of an additive such as hydroxybenzotriazole) or a carbodiimide derivative.

The term "combination of reagents," means a chemical reagent, or more than one reagent when necessary, that can be used to affect a desired chemical reaction. The choice of a particular reagent, or combination or reagents, will depend on factors that are familiar to those of ordinary skill in the art and include, but are not limited to, the identity of the reactants, the presence of other functional groups in the reactants, the solvent or solvents used in a particular chemical reaction, the temperature at which the chemical reaction will be performed, and the method or methods of purification of the desired chemical reaction product. The choice of a reagent, or combination of reagents, required to affect a particular

chemical reaction are within the knowledge of one of ordinary skill in the art and such a choice can be made without undue experimentation.

The term “base,” as used herein, means a so-called Bronsted-Lowry base. A Bronsted-Lowry base is a reagent that is capable of accepting a proton (H^+) from an acid present in a reaction mixture. Examples of Bronsted-Lowry bases include, but are not limited to, inorganic bases such as sodium carbonate, sodium bicarbonate, sodium hydroxide, potassium carbonate, potassium bicarbonate, potassium hydroxide, and cesium carbonate, inorganic bases such as triethylamine, diisopropylethylamine, diisopropylamine, dicyclohexylamine, morpholine, pyrrolidone, piperidine, pyridine, 4-N,N-dimethylaminopyridine (DMAP), and imidazole.

The term “chiral, non-racemic base,” as used herein, means a basic compound that can exist in an enantiomeric form and is not present in an equal amount with its corresponding opposite enantiomer. For example, the compound 2-phenylglycinol exists as two enantiomers of opposite configuration, the so-called (R)- and (S)-enantiomers. If the (R)- and the (S)-enantiomers are present in equal amounts, such a mixture is said to be “racemic.” If, however, one enantiomer is present in an amount greater than the other, the mixture is said to be “non-racemic.”

The term "stereoisomers" refers to compounds that have identical chemical constitution, but differ with regard to the arrangement of their atoms or groups in space. In particular, the term "enantiomers" refers to two stereoisomers of a compound that are non-superimposable mirror images of one another. The terms “racemic” or “racemic mixture,” as used herein, refer to a 1:1 mixture of enantiomers of a particular compound. The term "diastereomers", on the other hand, refers to the relationship between a pair of stereoisomers that comprise two or more asymmetric centers and are not mirror images of one another.

The term "stereochemically-enriched" product, when used herein, refers to a reaction product wherein a particular stereoisomer is present in a statistically significant greater amount relative to the other possible stereoisomeric products. For example, a product that comprises more of one enantiomer than the other would constitute a stereochemically enriched product. Similarly, a product that comprises more of one diastereoisomer than others would also constitute a stereochemically enriched product. The methods and processes contained herein are said to afford a “stereochemically enriched “ product. In such cases, the methods and processes contained herein begin with a mixture of stereoisomeric compounds in which all possible stereoisomers are present in about an equal amount and afford a product in which at least one stereoisomer is present in a statistically significant greater amount than the others.

The term “diastereomeric,” as used herein refers to the relationship between a pair of stereoisomers that comprise two or more asymmetric centers and are non-superimposable mirror images of one another. The phrases “diastereomeric salt,” or “diastereomeric salts,” as

used herein means a salt of a diastereomeric compound, wherein “diastereomer” is as defined herein.

The term “racemic,” as used herein, means a composition comprising a 1:1 ratio of enantiomers. The term “scalemic,” as used herein, means a composition comprising an unequal amount of enantiomers. For example, a composition comprising a 1:1 mixture of the (R)- and (S)-enantiomers of a compound of the present invention is termed a racemic composition or mixture. As an additional example, a composition comprising a 2:1 mixture of (R)- and (S)-enantiomers of a compound of the present invention is termed a scalemic composition or mixture. It is specifically contemplated that the methods of the present invention may be advantageously used to prepare a scalemic compound of the present invention from a racemic compound of the present invention.

The terms "resolution" and “resolving” mean a method of physically separating stereoisomeric compounds from a mixture of stereoisomers, such as a racemic mixture comprising two enantiomers of a particular compound. As used herein, “resolution” and “resolving” are meant to include both partial and complete resolution.

The terms “separating” or “separated,” as used herein, mean a process of physically isolating at least two different chemical compounds from each other. For example, if a chemical reaction takes place and produces at least two products, (A) and (B), the process of isolating both (A) and (B) from each other is termed “separating” (A) and (B). It is specifically contemplated that the separations of the present invention may be partial or complete as determined by analytical techniques known to those of ordinary skill in the art and those described herein.

The term “converting,” as used herein, means allowing a chemical reaction to take place with a starting material or materials to produce a different chemical product. For example, if chemical reactants (A) and (B) are allowed to react with each other to produce product (C), starting materials (A) and (B) can be said to have “converted” to product (C), or it can be said that (A) was “converted” to (C), or that (B) was “converted” to (C).

The term “substituted,” means that the specified group or moiety bears one or more substituents. The term “unsubstituted,” means that the specified group bears no substituents. The term “optionally substituted” means that the specified group is unsubstituted or substituted by one or more substituents.

Brief Description of the Figures

Figure 1 provides HIV-1 single-cycle infection and virus production assays. In the single-cycle infection assay (A), HeLa CD4/LTRBGal cells or MT-2 target cells are infected with either a VSV/HIVLuc or NL4/HIVLuc single-cycle infectious reporter virus in the presence or absence of compound. Viral infection of the HeLa CD4/LTRBGal cells is monitored by measuring HIV-1 Tat-mediated induction of the Beta-Galactosidase reporter gene present in the target cells as described in Materials and Methods. Alternatively, viral infection of the MT-2 cells is monitored by measuring the expression of the firefly luciferase reporter gene

encoded by the virus. Single-cycle infection assays are sensitive to early stage inhibitors (e.g., NNRTI, NRTI and INI) and entry inhibitors when cells are infected with the HIV/HIVLuc virus. In the virus production assay (B), an envelope deleted NL4-3 reporter virus cDNA (pNL4-3deltaEnv) is co-transfected into HEK 293 cells with either a VSV-G expression vector, an HIV envelope expression vector, or both vectors in the presence or absence of compound. Infectious virus production is then measured by quantifying the expression of the firefly luciferase reporter gene encoded by the virus as described in Materials and Methods. Virus production assays are sensitive to late stage inhibitors (e.g., protease inhibitors).

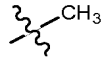
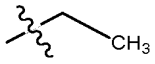
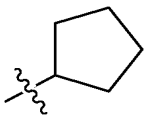
Figure 2 depicts an HIV envelope (Env) expression assay. In (I), an HIV Tat/Env expression vector (pNL4Env) was transfected into HEK 293 cells and transfected cells were then either treated with compound or incubated in the absence of compound treatment for 24 hours. In (II), 24 hours after the transfection, the transfected cells were co-cultured with HelaCD4/LTRBGal target cells in the presence or absence of compound. Functional envelope expression was then measured by monitoring induction of Beta-Galactosidase expression in the target cells.

Figure 3 depicts the compound 1 inhibition of HIV-1 envelope processing in transfected cells. HEK 293 cells were transfected with pNL4-3, pNL4/IIIB, or mock transfected in the presence of various concentrations of compound 1 (denoted above each lane) or the absence of compound. 48 hours after transfection, transfected cells or mock–-transfected cells were harvested and extracts of the harvested cells were subjected to a Western analysis using either gp120 antiserum (A) or p24 antiserum (B). In addition, infectious virus production was measured in the supernatants of transfected cells as described in Materials and Methods. Percent inhibition of infectious virus production relative to the no compound control is shown directly under each lane. The Western analyses were quantified by densitometer scanning and the relative amounts of gp160 as a percentage of the total HIV-1 envelope protein are shown at the bottom of Figure 3A, while the relative levels of HIV-1 p24 protein as a percentage of that observed for the no compound control are shown at the bottom of Figure 3B.

Figure 4 depicts a Western analysis performed using a gp120 antibody and isolated virions produced in the presence or absence of 2X EC_{90} concentrations of compound 1 or NFV. HEK 293 cells were transfected with pNL4-3 or mock transfected in the presence or absence of 2x EC_{90} concentrations of compound I or NFV. 48 hours after transfection, supernatants of transfected cells or mock -transfected cells were harvested and virions isolated. Isolated virions were then subjected to a Western analysis using either gp120 antiserum or p24 antiserum (denoted at the bottom of the figure).

Detailed Description

In accordance with a convention used in the art, the symbol  is used in structural formulas herein to depict the bond that is the point of attachment of the moiety or substituent

to the core or backbone structure. In accordance with another convention, in some structural formulae herein the carbon atoms and their bound hydrogen atoms are not explicitly depicted, e.g.,  represents a methyl group,  represents an ethyl group,  represents a cyclopentyl group, etc.

The compounds of the present invention may have asymmetric carbon atoms. The carbon-carbon bonds of the compounds of the present invention may be depicted herein using a solid line (———), a solid wedge (◀), or a dotted wedge (⋯). The use of a solid line to depict bonds to asymmetric carbon atoms is meant to indicate that all possible stereoisomers at that carbon atom are included. The use of either a solid or dotted wedge to depict bonds to asymmetric carbon atoms is meant to indicate that only the stereoisomer shown is meant to be included. It is possible that compounds of the invention may contain more than one asymmetric carbon atom. In those compounds, the use of a solid line to depict bonds to asymmetric carbon atoms is meant to indicate that all possible stereoisomers are meant to be included. The use of a solid line to depict bonds to one or more asymmetric carbon atoms in a compound of the invention and the use of a solid or dotted wedge to depict bonds to other asymmetric carbon atoms in the same compound is meant to indicate that a mixture of diastereomers is present.

Solutions of individual stereoisomeric compounds of the present invention may rotate plane-polarized light. The use of either a “(+)” or “(-)” symbol in the name of a compound of the invention indicates that a solution of a particular stereoisomer rotates plane-polarized light in the (+) or (-) direction, as measured using techniques known to those of ordinary skill in the art.

Diastereomeric mixtures can be separated into their individual diastereomers on the basis of their physical chemical differences by methods known to those skilled in the art, for example, by chromatography or fractional crystallization. Enantiomers can be separated by converting the enantiomeric mixtures into a diastereomeric mixture by reaction with an appropriate optically active compound (e.g., alcohol), separating the diastereomers and converting (e.g., hydrolyzing) the individual diastereomers to the corresponding pure enantiomers. All such isomers, including diastereomeric mixtures and pure enantiomers are considered as part of the invention.

Alternatively, individual stereoisomeric compounds of the present invention may be prepared in enantiomerically enriched form by asymmetric synthesis. Asymmetric synthesis may be performed using techniques known to those of skill in the art, such as the use of asymmetric starting materials that are commercially available or readily prepared using methods known to those of ordinary skill in the art, the use of asymmetric auxiliaries that may

be removed at the completion of the synthesis, or the resolution of intermediate compounds using enzymatic methods. The choice of such a method will depend on factors that include, but are not limited to, the availability of starting materials, the relative efficiency of a method, and whether such methods are useful for the compounds of the invention containing particular functional groups. Such choices are within the knowledge of one of ordinary skill in the art.

When the compounds of the present invention contain asymmetric carbon atoms, the derivative salts, prodrugs and solvates may exist as single stereoisomers, racemates, and/or mixtures of enantiomers and/or diastereomers. All such single stereoisomers, racemates, and mixtures thereof are intended to be within the scope of the present invention.

As generally understood by those skilled in the art, an optically pure compound is one that is enantiomerically pure. As used herein, the term "optically pure" is intended to mean a compound comprising at least a sufficient activity. Preferably, an optically pure amount of a single enantiomer to yield a compound having the desired pharmacological pure compound of the invention comprises at least 90% of a single isomer (80% enantiomeric excess), more preferably at least 95% (90% e.e.), even more preferably at least 97.5% (95% e.e.), and most preferably at least 99% (98% e.e.).

If a derivative used in the method of the invention is a base, a desired salt may be prepared by any suitable method known to the art, including treatment of the free base with an inorganic acid, such as: hydrochloric acid; hydrobromic acid; sulfuric acid; nitric acid; phosphoric acid; and the like, or with an organic acid, such as: acetic acid; maleic acid; succinic acid; mandelic acid; fumaric acid; malonic acid; pyruvic acid; oxalic acid; glycolic acid; salicylic acid; pyranosidyl acid, such as glucuronic acid or galacturonic acid; alpha-hydroxy acid, such as citric acid or tartaric acid; amino acid, such as aspartic acid or glutamic acid; aromatic acid, such as benzoic acid or cinnamic acid; sulfonic acid, such as p-toluenesulfonic acid or ethanesulfonic acid; and the like.

If a derivative used in the method of the invention is an acid, a desired salt may be prepared by any suitable method known to the art, including treatment of the free acid with an inorganic or organic base, such as an amine (primary, secondary, or tertiary); an alkali metal or alkaline earth metal hydroxide; or the like. Illustrative Examples of suitable salts include organic salts derived from amino acids such as glycine and arginine; ammonia; primary, secondary, and tertiary amines; and cyclic amines, such as piperidine, morpholine, and piperazine; as well as inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum, and lithium.

In the case of derivatives, prodrugs, salts, or solvates that are solids, it is understood by those skilled in the art that the derivatives, prodrugs, salts, and solvates used in the method of the invention, may exist in different polymorph or crystal forms, all of which are intended to be within the scope of the present invention and specified formulas. In addition, the derivative, salts, prodrugs and solvates used in the method of the invention may exist as tautomers, all of which are intended to be within the broad scope of the present invention.

The compounds of the present invention that are basic in nature are capable of forming a wide variety of different salts with various inorganic and organic acids. Although such salts must be pharmaceutically acceptable for administration to animals, it is often desirable in practice to initially isolate the compound of the present invention from the reaction mixture as a pharmaceutically unacceptable salt and then simply convert the latter back to the free base compound by treatment with an alkaline reagent and subsequently convert the latter free base to a pharmaceutically acceptable acid addition salt. The acid addition salts of the base compounds of this invention are readily prepared by treating the base compound with a substantially equivalent amount of the chosen mineral or organic acid in an aqueous solvent medium or in a suitable organic solvent, such as methanol or ethanol. Upon careful evaporation of the solvent, the desired solid salt is readily obtained. The desired acid salt can also be precipitated from a solution of the free base in an organic solvent by adding to the solution an appropriate mineral or organic acid.

Those compounds of the present invention that are acidic in nature are capable of forming base salts with various pharmacologically acceptable cations. Examples of such salts include the alkali metal or alkaline-earth metal salts and particularly, the sodium and potassium salts. These salts are all prepared by conventional techniques. The chemical bases which are used as reagents to prepare the pharmaceutically acceptable base salts of this invention are those which form non-toxic base salts with the acidic compounds of the present invention. Such non-toxic base salts include those derived from such pharmacologically acceptable cations as sodium, potassium calcium and magnesium, etc. These salts can easily be prepared by treating the corresponding acidic compounds with an aqueous solution containing the desired pharmacologically acceptable cations, and then evaporating the resulting solution to dryness, preferably under reduced pressure. Alternatively, they may also be prepared by mixing lower alkanolic solutions of the acidic compounds and the desired alkali metal alkoxide together, and then evaporating the resulting solution to dryness in the same manner as before. In either case, stoichiometric quantities of reagents are preferably employed in order to ensure completeness of reaction and maximum yields of the desired final product.

The activity of the compounds as inhibitors of HIV activity may be measured by any of the suitable methods available in the art, including *in vivo* and *in vitro* assays. Examples of suitable assays for activity measurements are described herein.

Administration of the compounds and their pharmaceutically acceptable prodrugs, salts, active metabolites, and solvates may be performed according to any of the accepted modes of administration available to those skilled in the art. Illustrative Examples of suitable modes of administration include oral, nasal, parenteral, topical, transdermal, and rectal. Oral and intravenous deliveries are preferred.

An HIV-inhibiting agent of the present invention may be administered as a pharmaceutical composition in any suitable pharmaceutical form. Suitable pharmaceutical forms include solid, semisolid, liquid, or lyopholized formulations, such as tablets, powders,

capsules, suppositories, suspensions, liposomes, and aerosols. The HIV-inhibiting agent may be prepared as a solution using any of a variety of methodologies. For Example, the HIV-inhibiting agent can be dissolved with acid (e.g., 1 M HCl) and diluted with a sufficient volume of a solution of 5% dextrose in water (D5W) to yield the desired final concentration of HIV-inhibiting agent (e.g., about 15 mM). Alternatively, a solution of D5W containing about 15 mM HCl can be used to provide a solution of the HIV-inhibiting agent at the appropriate concentration. Further, the HIV-inhibiting agent can be prepared as a suspension using, for example, a 1% solution of carboxymethylcellulose (CMC).

Acceptable methods of preparing suitable pharmaceutical forms of the pharmaceutical compositions are known or may be routinely determined by those skilled in the art. For Example, pharmaceutical preparations may be prepared following conventional techniques of the pharmaceutical chemist involving steps such as mixing, granulating, and compressing when necessary for tablet forms, or mixing, filling, and dissolving the ingredients as appropriate, to give the desired products for oral, parenteral, topical, intravaginal, intranasal, intrabronchial, intraocular, intraaural, and/or rectal administration.

Pharmaceutical compositions of the invention may also include suitable excipients, diluents, vehicles, and carriers, as well as other pharmaceutically active agents, depending upon the intended use. Solid or liquid pharmaceutically acceptable carriers, diluents, vehicles, or excipients may be employed in the pharmaceutical compositions. Illustrative solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, sucrose, talc, gelatin, pectin, acacia, magnesium stearate, and stearic acid. Illustrative liquid carriers include syrup, peanut oil, olive oil, saline solution, and water. The carrier or diluent may include a suitable prolonged-release material, such as glyceryl monostearate or glyceryl distearate, alone or with a wax. When a liquid carrier is used, the preparation may be in the form of a syrup, elixir, emulsion, soft gelatin capsule, sterile injectable liquid (e.g., solution), or a nonaqueous or aqueous liquid suspension.

A dose of the pharmaceutical composition may contain at least a therapeutically effective amount of an HIV-inhibiting agent and preferably is made up of one or more pharmaceutical dosage units. The selected dose may be administered to a mammal, for example, a human, in need of treatment mediated by inhibition of HIV activity, by any known or suitable method of administering the dose, including topically, for example, as an ointment or cream; orally; rectally, for example, as a suppository; parenterally by injection; intravenously; or continuously by intravaginal, intranasal, intrabronchial, intraaural, or intraocular infusion. When the composition is administered in conjunction with a cytotoxic drug, the composition can be administered before, with, and/or after introduction of the cytotoxic drug. However, when the composition is administered in conjunction with radiotherapy, the composition is preferably introduced before radiotherapy is commenced.

Methods of preparing various pharmaceutical compositions with a specific amount of active compound are known, or will be apparent, to those skilled in this art. For examples, see

Remington's Pharmaceutical Sciences, Mack Publishing Company, Easter, Pa., 15th Edition (1975).

It will be appreciated that the actual dosages of the HIV-inhibiting agents used in the pharmaceutical compositions of this invention will be selected according to the properties of the particular agent being used, the particular composition formulated, the mode of administration and the particular site, and the host and condition being treated. Optimal dosages for a given set of conditions can be ascertained by those skilled in the art using conventional dosage-determination tests. For oral administration, e.g., a dose that may be employed is from about 0.001 to about 1000 mg/kg body weight, or from about 0.1 to about 100 mg/kg body weight, or from about 1 to about 50 mg/kg body weight, or from about 0.1 to about 1 mg/kg body weight, with courses of treatment repeated at appropriate intervals. The dosage forms of the pharmaceutical formulations described herein may contain an amount of a compound of the present invention, or a pharmaceutically acceptable salt of solvate thereof, deemed appropriate by one of ordinary skill in the art. For example, such dosage forms may contain from about 1 mg to about 1500 mg of a compound of the present invention, or may contain from about 5 mg to about 1500 mg, or from about 5 mg to about 1250 mg, or from about 10 mg to about 1250 mg, or from about 25 mg to about 1250 mg, or from about 25 mg to about 1000 mg, or from about 50 mg to about 1000 mg, or from about 50 mg to about 750 mg, or from about 75 mg to about 750 mg, or from about 100 mg to about 750 mg, or from about 125 mg to about 750 mg, or from about 150 mg to about 750 mg, or from about 150 mg to about 500 mg of a compound of the present invention, or a pharmaceutically acceptable salt or solvate thereof.

The subject invention also includes isotopically-labelled compounds, which are identical to those recited in the compounds of the present invention, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively. Compounds of the present invention, prodrugs thereof, and pharmaceutically acceptable salts of said compounds or of said prodrugs which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labelled compounds of the present invention, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labeled compounds of the present invention and prodrugs thereof

can generally be prepared by carrying out the procedures disclosed in the Schemes and/or in the Examples and Preparations below, by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent.

The compounds of the present invention are potent inhibitors of HIV virus, in particular HIV replication, and even in more particular, HIV virion maturation. The compounds are all adapted to therapeutic use as anti-HIV agents in mammals, particularly in humans.

The active compound may be applied as a sole therapy or may involve one or more other antiviral substances, for example those selected from, for example, HIV inhibitors such as nelfinavir, delavirdine, indinavir, nevirapine, saquinavir, and tenofovir. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate dosing of the individual components of the treatment.

The compounds of the present invention may be administered in combination with an additional agent or agents for the treatment of a mammal, such as a human, that is suffering from an infection with the HIV virus, AIDS, AIDS-related complex (ARC), or any other disease or condition which is related to infection with the HIV virus. The agents that may be used in combination with the compounds of the present invention include, but are not limited to, those useful as HIV protease inhibitors, HIV reverse transcriptase inhibitors, non-nucleoside HIV reverse transcriptase inhibitors, inhibitors of HIV integrase, CCR5 inhibitors, CXCR4 inhibitors, HIV fusion inhibitors, gp120 inhibitors, compounds useful as immunomodulators, compounds that inhibit the HIV virus by an unknown mechanism, compounds useful for the treatment of herpes viruses, compounds useful as anti-infectives, and others as described below.

Compounds useful as HIV protease inhibitors that may be used in combination with the compounds of the present invention include, but are not limited to, 141 W94 (amprenavir), CGP-73547, CGP-61755, DMP-450, nelfinavir, ritonavir, saquinavir (invirase), lopinavir, TMC-126, atazanavir, palinavir, GS-3333, KN I-413, KNI-272, LG-71350, CGP-61755, PD 173606, PD 177298, PD 178390, PD 178392, U-140690, ABT-378, DMP-450, AG-1776, MK-944, VX-478, indinavir, tipranavir, TMC-114, DPC-681, DPC-684, fosamprenavir calcium (Lexiva), benzenesulfonamide derivatives disclosed in WO 03053435, R-944, Ro-03-34649, VX-385, GS-224338, OPT-TL3, PL-100, SM-309515, AG-148, DG-35-VIII, DMP-850, GW-5950X, KNI-1039, L-756423, LB-71262, LP-130, RS-344, SE-063, UIC-94-003, Vb-19038, A-77003, BMS-182193, BMS-186318, SM-309515, JE-2147, GS-9005.

Compounds useful as inhibitors of the HIV reverse transcriptase enzyme that may be used in combination with the compounds of the present invention include, but are not limited to, abacavir, FTC, GS-840, lamivudine, adefovir dipivoxil, beta-fluoro-ddA, zalcitabine, didanosine, stavudine, zidovudine, tenofovir, amdoxovir, SPD-754, SPD-756, racivir, reverset (DPC-817), MIV-210 (FLG), beta-L-Fd4C (ACH-126443), MIV-310 (alovudine, FLT), dOTC, DAPD, entecavir, GS-7340, emtricitabine and alovudine.

Compounds useful as non-nucleoside inhibitors of the HIV reverse transcriptase enzyme include, but are not limited to, efavirenz, HBY-097, nevirapine, TMC-120 (dapivirine),

TMC-125, etravirine, delavirdine, DPC-083, DPC-961, TMC-120, capravirine, GW-678248, GW-695634, calanolide, and tricyclic pyrimidinone derivatives as disclosed in WO 03062238.

Compounds useful as CCR5 inhibitors that may be used in combination with the compounds of the present invention include, but are not limited to, TAK-779, SC-351125, SCH-D, UK-427857, PRO-140, and GW-873140 (Ono-4128, AK-602).

Compounds useful as inhibitors of HIV integrase enzyme that may be used in combination with the compounds of the present invention include, but are not limited to, GW-810781, L-000870810 (Merck), 1,5-naphthyridine-3-carboxamide derivatives disclosed in WO 03062204, compounds disclosed in WO 03047564, compounds disclosed in WO 03049690, and 5-hydroxypyrimidine-4-carboxamide derivatives disclosed in WO 03035076.

Fusion inhibitors for the treatment of HIV that may be used in combination with the compounds of the present invention include, but are not limited to, enfuvirtide (T-20), T-1249, and fused tricyclic compounds disclosed in JP 2003171381.

CXCR4 inhibitors for the treatment of HIV that may be used in combination with the compounds of the present invention include, but are not limited to AMD3100, and AMD070.

Gp120 inhibitors for the treatment of HIV that may be used in combination with the compounds of the present invention include, but are not limited to BMS-378806 and BMS-488043.

Other compounds that are useful inhibitors of HIV that may be used in combination with the compounds of the present invention include, but are not limited to, Soluble CD4, TNX-355, PRO-542, tenofovir disoproxil fumarate, and compounds disclosed in JP 2003119137.

Compounds useful in the treatment or management of infection from viruses other than HIV that may be used in combination with the compounds of the present invention include, but are not limited to, acyclovir, fomivirsen, penciclovir, HPMPC, oxetanocin G, AL-721, cidofovir, cytomegalovirus immune globin, cytovene, fomivganciclovir, famciclovir, foscarnet sodium, Isis 2922, KNI-272, valacyclovir, virazole ribavirin, valganciclovir, ME-609, and PCL-016.

Compounds that act as immunomodulators and may be used in combination with the compounds of the present invention include, but are not limited to, AD-439, AD-519, Alpha Interferon, AS-101, bropirimine, acemannan, CL246,738, EL10, FP-21399, gamma interferon, granulocyte macrophage colony stimulating factor, IL-2, immune globulin intravenous, IMREG-1, IMREG-2, imuthiol diethyl dithio carbamate, alpha-2 interferon, methionine-enkephalin, MTP-PE, granulocyte colony stimulating sactor, remune, rCD4, recombinant soluble human CD4, interferon alfa-2, SK&F106528, soluble T4 yhymopentin, tumor necrosis factor (TNF), tucaresol, recombinant human interferon beta, and interferon alfa n-3.

Anti-infectives that may be used in combination with the compounds of the present invention include, but are not limited to, atovaquone, azithromycin, clarithromycin, trimethoprim, trovafloxacin, pyrimethamine, daunorubicin, clindamycin with primaquine,

fluconazole, pastill, ornidyl, eflornithine pentamidine, rifabutin, spiramycin, intraconazole-R51211, trimetrexate, daunorubicin, recombinant human erythropoietin, recombinant human growth hormone, megestrol acetate, testerone, and total enteral nutrition.

Antifungals that may be used in combination with the compounds of the present invention include, but are not limited to, anidulafungin, C31G, caspofungin, DB-289, fluconzaole, itraconazole, ketoconazole, micafungin, posaconazole, and voriconazole.

Other compounds that may be used in combination with the compounds of the present invention include, but are not limited to, acmannan, ansamycin, LM 427, AR177, BMS-232623, BMS-234475, CI-1012, curdlan sulfate, dextran sulfate, STOCRINE EL10, hypericin, lobucavir, novapren, peptide T octabpeptide sequence, trisodium phosphonoformate, probucol, and RBC-CD4.

In addition, the compounds of the present invention may be used in combination with anti-proliferative agents for the treatment of conditions such as Kaposi's sarcoma. Such agents include, but are not limited to, inhibitors of metallo-matrix proteases, A-007, bevacizumab, BMS-275291, halofuginone, interleukin-12, rituximab, paclitaxel, porfimer sodium, rebimastat, and COL-3.

The particular choice of an additional agent or agents will depend on a number of factors that include, but are not limited to, the condition of the mammal being treated, the particular condition or conditions being treated, the identity of the compound or compounds of the present invention and the additional agent or agents, and the identity of any additional compounds that are being used to treat the mammal. The particular choice of the compound or compounds of the invention and the additional agent or agents is within the knowledge of one of ordinary skill in the art.

The compounds of the present invention may be administered in combination with any of the above additional agents for the treatment of a mammal, such as a human, that is suffering from an infection with the HIV virus, AIDS, AIDS-related complex (ARC), or any other disease or condition which is related to infection with the HIV virus. Such a combination may be administered to a mammal such that a compound or compounds of the present invention are present in the same formulation as the additional agents described above. Alternatively, such a combination may be administered to a mammal suffering from infection with the HIV virus such that the compound or compounds of the present invention are present in a formulation that is separate from the formulation in which the additional agent is found. If the compound or compounds of the present invention are administered separately from the additional agent, such administration may take place concomitantly or sequentially with an appropriate period of time in between. The choice of whether to include the compound or compounds of the present invention in the same formulation as the additional agent or agents is within the knowledge of one of ordinary skill in the art.

Additionally, the compounds of the present invention may be administered to a mammal, such as a human, in combination with an additional agent that has the effect of

increasing the exposure of the mammal to a compound of the invention. The term “exposure,” as used herein, refers to the concentration of a compound of the invention in the plasma of a mammal as measured over a period of time. The exposure of a mammal to a particular compound can be measured by administering a compound of the invention to a mammal in an appropriate form, withdrawing plasma samples at predetermined times, and measuring the amount of a compound of the invention in the plasma using an appropriate analytical technique, such as liquid chromatography or liquid chromatography/mass spectroscopy. The amount of a compound of the invention present in the plasma at a certain time is determined and the concentration and time data from all the samples are plotted to afford a curve. The area under this curve is calculated and affords the exposure of the mammal to the compound. The terms “exposure,” “area under the curve,” and “area under the concentration/time curve” are intended to have the same meaning and may be used interchangeably throughout.

Among the agents that may be used to increase the exposure of a mammal to a compound of the present invention are those that can as inhibitors of at least one isoform of the cytochrome P450 (CYP450)enzymes. The isoforms of CYP450 that may be beneficially inhibited include, but are not limited to, CYP1A2, CYP2D6, CYP2C9, CYP2C19 and CYP3A4. Suitable agents that may be used to inhibit CYP 3A4 include, but are not limited to, ritonavir.

Such a combination may be administered to a mammal such that a compound or compounds of the present invention are present in the same formulation as the additional agents described above. Alternatively, such a combination may be administered such that the compound or compounds of the present invention are present in a formulation that is separate from the formulation in which the additional agent is found. If the compound or compounds of the present invention are administered separately from the additional agent, such administration may take place concomitantly or sequentially with an appropriate period of time in between. The choice of whether to include the compound or compounds of the present invention in the same formulation as the additional agent or agents is within the knowledge of one of ordinary skill in the art.

Suitable bases for use in these reactions include inorganic bases and organic bases. Suitable inorganic bases include, but are not limited to, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, sodium hydroxide, sodium hydride, potassium hydride, and cesium carbonate. Preferably, the base is potassium carbonate. Suitable organic bases include, but are not limited to, pyridine, triethylamine, tributylamine, triethanolamine, N-methylmorpholine, N-ethyl-N,N-diisopropylamine, DBU, and 4-N,N-dimethylaminopyridine. These reactions can also be performed in the presence of a catalytic amount of a suitable acid. Suitable acids include both Bronsted-Lowry and Lewis acids. Furthermore, these reactions are generally performed in a solvent or mixture of solvents that will not interfere with desired chemical reaction. Furthermore, appropriate solvents include those that are known to those of skill in the art to be compatible with the reaction conditions and include alkyl esters and aryl esters, alkyl, heterocyclic, and aryl

ethers, hydrocarbons, alkyl and aryl alcohols, alkyl and aryl halogenated compounds, alkyl or aryl nitriles, alkyl and aryl ketones, and non-protic heterocyclic solvents. For example, suitable solvents include, but are not limited to, ethyl acetate, isobutyl acetate, isopropyl acetate, n-butyl acetate, methyl isobutyl ketone, dimethoxyethane, diisopropyl ether, chlorobenzene, dimethyl formamide, dimethyl acetamide, propionitrile, butyronitrile, t-amyl alcohol, acetic acid, diethyl ether, methyl-t-butyl ether, diphenyl ether, methylphenyl ether, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, pentane, hexane, heptane, methanol, ethanol, 1-propanol, 2-propanol, t-butanol, n-butanol, 2-butanol, dichloromethane, chloroform, 1,2-dichloroethane, acetonitrile, benzonitrile, acetone, 2-butanone, benzene, toluene, anisole, xylenes, and pyridine, or any mixture of the above solvents. Additionally, water may be used as a co-solvent if it will not interfere with the desired transformation. Finally, such reactions can be performed at a temperature in the range of from about 0 °C to about 100 °C, or in the range of from about 25 °C to about 100 °C, or in the range of from about 35 °C to about 75 °C, or in the range of from about 45 °C to about 55 °C, or at about 50 °C. The choice of a particular reducing agent, solvent, and temperature will depend on several factors including, but not limited to, the identity of the particular reactants and the functional groups present in such reactants. Such choices are within the knowledge of one of ordinary skill in the art and can be made without undue experimentation.

Such reactions may be performed using a suitable base in a suitable solvent. Suitable bases include, but are not limited to, potassium carbonate, sodium carbonate, potassium bicarbonate, sodium bicarbonate, potassium hydroxide, and sodium hydroxide. Solvents that may be used include, but are not limited to, methyl alcohol, ethyl alcohol, iso-propyl alcohol, n-propyl alcohol, acetonitrile, and DMF, or a mixture of them. Additionally, water may be used as a cosolvent if necessary. These reactions may be performed at a temperature of from about 0 °C to about 150 °C. The particular choice of a base or combination of bases, solvent or combination of solvents, and reaction temperature will depend on the particular starting material being used and such choices are within the knowledge of one of ordinary skill in the art and can be made without undue experimentation.

These reactions are generally performed in the presence of a reducing agent, such as a borane source or hydrogen in the presence of suitable catalyst. Suitable borane sources include, but are not limited to, borane-trimethylamine complex, borane-dimethylamine complex, borane t-butyl amine complex, and borane-pyrdine complex. Suitable catalysts for use in the presence of a reducing agent such as hydrogen include, but are not limited to, nickel, palladium, rhodium and ruthenium. Furthermore, such reactions are performed in a solvent or mixture of solvents that will not interfere with desired chemical reaction. Furthermore, appropriate solvents include those that are known to those of skill in the art to be compatible with the reaction conditions and include alkyl esters and aryl esters, alkyl, heterocyclic, and aryl ethers, hydrocarbons, alkyl and aryl alcohols, alkyl and aryl halogenated compounds, alkyl or aryl nitriles, alkyl and aryl ketones, and non-protic

heterocyclic solvents. For example, suitable solvents include, but are not limited to, ethyl acetate, isobutyl acetate, isopropyl acetate, n-butyl acetate, methyl isobutyl ketone, dimethoxyethane, diisopropyl ether, chlorobenzene, dimethyl formamide, dimethyl acetamide, propionitrile, butyronitrile, t-amyl alcohol, acetic acid, diethyl ether, methyl-t-butyl ether, diphenyl ether, methylphenyl ether, tetrahydrofuran, 2-methyltetrahydrofuran, 1,4-dioxane, pentane, hexane, heptane, methanol, ethanol, 1-propanol, 2-propanol, t-butanol, n-butanol, 2-butanol, dichloromethane, chloroform, 1,2-dichloroethane, acetonitrile, benzonitrile, benzene, toluene, anisole, xylenes, and pyridine, or any mixture of the above solvents. Additionally, water may be used as a co-solvent if it will not interfere with the desired transformation. Finally, such reactions can be performed at a temperature in the range of from about 0 °C to about 75 °C, preferably in the range of from about 0 °C to about 32 °C, most preferably at room or ambient temperature. The choice of a particular reducing agent, solvent, and temperature will depend on several factors including, but not limited to, the identity of the particular reactants and the functional groups present in such reactants. Such choices are within the knowledge of one of ordinary skill in the art and can be made without undue experimentation.

The following Examples are meant to illustrate particular embodiments of the present invention only and are not intended to limit its scope in any manner.

Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about". Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Examples

In the examples described below, unless otherwise indicated, all temperatures in the following description are in degrees Celsius (°C) and all parts and percentages are by weight, unless indicated otherwise.

Various starting materials and other reagents were purchased from commercial suppliers, such as Aldrich Chemical Company or Lancaster Synthesis Ltd., and used without further purification, unless otherwise indicated. HeLa, CEM-SS, MT-2, PM1, C8166, CEM4, 174XCEM and HEK 293 cells were obtained through the National Institutes of Health AIDS Research and Reference Reagent Program (Bethesda, MD). HeLa and HEK 293 cells were propagated in Dulbeccos's Modified Eagle Medium (DMEM; Life Technologies, Gaithersburg MD) containing 10% fetal bovine serum (FBS; HyClone, Logan, UT). CEM-SS, MT-2, PM1, C8166, CEM4, and 174XCEM cells were propagated in RPMI medium (Life Technologies)

containing 10% fetal bovine serum (FBS; HyClone). The HIV-1 IIIB and HIV-1Ba-L viruses and the pNL4-3 HIV-1 infectious molecular clone were also obtained through the National Institutes of Health AIDS Research and Reference Reagent Program.

The reactions set forth below were performed under a positive pressure of nitrogen, argon or with a drying tube, at ambient temperature (unless otherwise stated), in anhydrous solvents. Analytical thin-layer chromatography was performed on glass-backed silica gel 60°F 254 plates (Analtech (0.25 mm)) and eluted with the appropriate solvent ratios (v/v). The reactions were assayed by high-pressure liquid chromatography (HPLC) or thin-layer chromatography (TLC) and terminated as judged by the consumption of starting material. The TLC plates were visualized by UV, phosphomolybdic acid stain, or iodine stain.

^{1}H-NMR spectra were recorded on a Bruker instrument operating at 300 MHz or 400 MHz and ^{13}C-NMR spectra were recorded at 75 MHz. NMR spectra are obtained as DMSO-d_6 or $CDCl_3$ solutions (reported in ppm), using chloroform as the reference standard (7.25 ppm and 77.00 ppm) or DMSO-d_6 (2.50 ppm and 39.52 ppm). Other NMR solvents were used as needed. When peak multiplicities are reported, the following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broadened, dd = doublet of doublets, dt = doublet of triplets. Coupling constants, when given, are reported in Hertz.

Infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrometer as neat oils, as KBr pellets, or as $CDCl_3$ solutions, and when reported are in wave numbers (cm^{-1}). The mass spectra were obtained using LC/MS or APCI. All melting points are uncorrected.

In the following examples and preparations, “Et" means ethyl, “Ac” means acetyl, “Me” means methyl, "Ph" means phenyl, “$(PhO)_2POCl$” means chlorodiphenylphosphate, "HCl" means hydrochloric acid, "EtOAc" means ethyl acetate, "Na_2CO_3" means sodium carbonate, "NaOH" means sodium hydroxide, "NaCl" means sodium chloride, "NEt_3" means triethylamine , "THF" means tetrahydrofuran, "DIC" means diisopropylcarbodiimide, "HOBt" means hydroxy benzotriazole, "H_2O" means water, "$NaHCO_3$" means sodium hydrogen carbonate, ”K_2CO_3" means potassium carbonate, "MeOH" means methanol, "i-PrOAc" means isopropyl acetate, "$MgSO_4$" means magnesium sulfate, "DMSO" means dimethylsulfoxide, "AcCl" means acetyl chloride, "CH_2Cl_2" means methylene chloride, "MTBE" means methyl t-butyl ether, "DMF" means dimethyl formamide, "$SOCl_2$" means thionyl chloride, ”H_3PO_4" means phosphoric acid, "CH_3SO_3H" means methanesulfonic acid, " Ac_2O” means acetic anhydride, “CH_3CN” means acetonitrile, “KOH” means potassium hydroxide, “CDI” means carbonyl diimidazole, “DABCO” means 1,4-diazabicyclo[2.2.2]octane, “IPE” means isopropyl ether, “MTBE” means methyl tert-butyl ether, “Et_2O” means diethylether, “Na_2SO_4“ means sodium sulfate, “NBS” means N-bromosuccinimide, “TEA” means triethylamine, “DCM” means dichloromethane, “TBAB” means tetrabutylammonium bromide, “HMPA” means hexamethylphosphoramide, “NMP” means 1-methyl-2-pyrrolidinone, “DMAC” means N,N-dimethylacetamide, “h” means hours, “min” means minutes, “mol” means moles, “rt” means room temperature, “ml” means milliliter, “μM” means micromolar, “nM” means nanomolar,

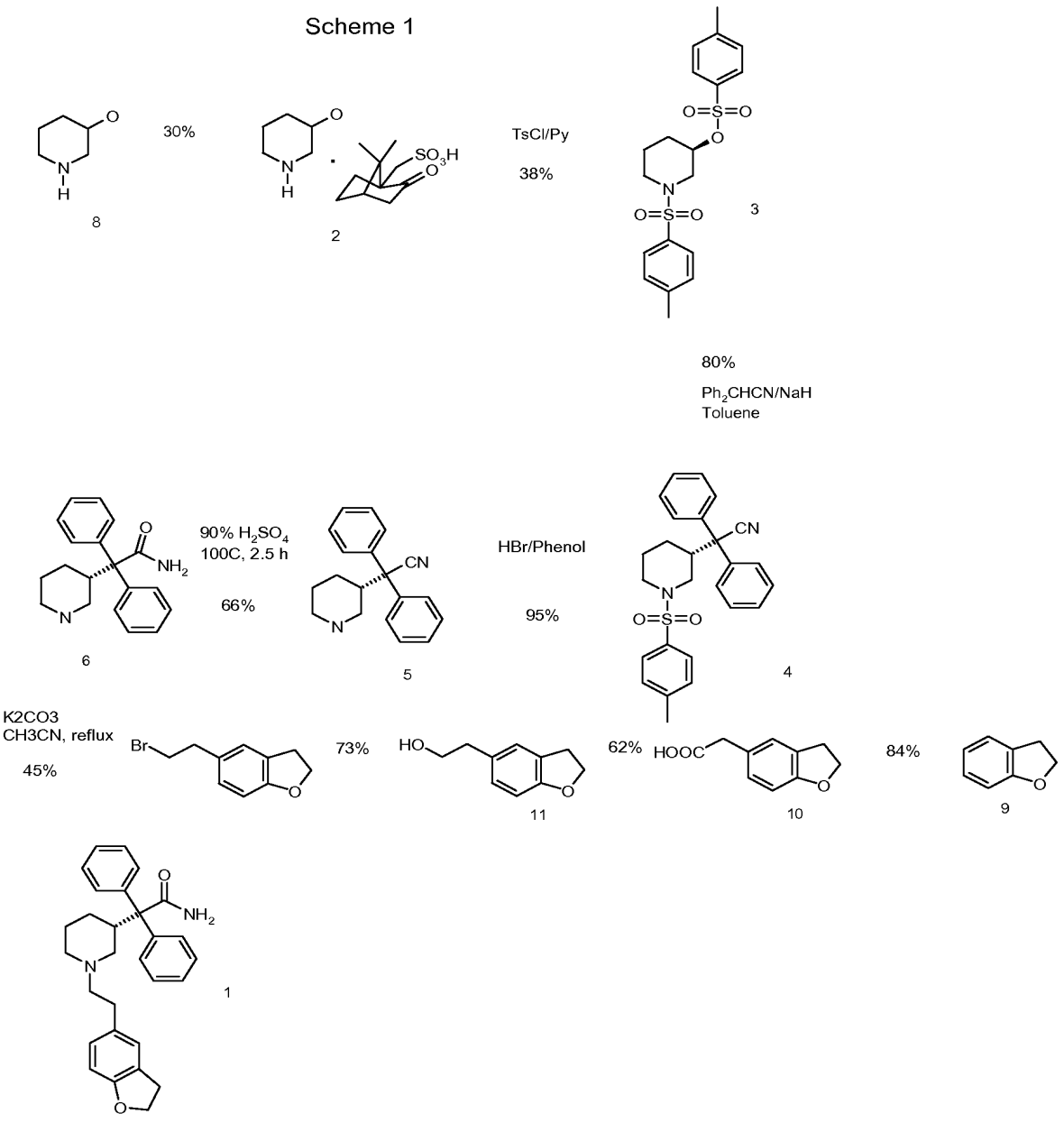
"DMEM" means Dulbeccos's Modified Eagle Medium, "PBS" means phosphate buffered saline, "FBS" means fetal bovine serum, "PCR" means polymerase chain reaction, "HEK" means human embryonic kidney, "moi" means multiplicity of infection, "$TCID_{50}$" means 50% tissue culture infectious dose, "RT" means reverse transcriptase, "IN" means integrase, "NNRTI" means non-nucleoside reverse transcriptase inhibitor, "NRTI" means nucleoside reverse transcriptase inhibitor, "INI" means integrase inhibitor, "PI" means protease inhibitor, "EFV" means efavirenz, "NFV" means nelfinavir, "ATA" means aurintricarboxylic acid, and "nt" means nucleotide.

In general, the compounds of the present invention may be prepared according to the methods described herein, as well as methods known to those of ordinary skill in the art. The methods described herein are not meant to, and should not be construed to, limit the scope of the present invention in any way.

Compounds of the present invention may also be prepared according to the methods described in United States Patent No. 5,422,358, and PCT Publication No. WO90/05133, the entire disclosures of which are incorporated herein.

A general scheme for the preparation of compounds of the invention is shown below starting from commercially available compound piperidine-3-ol (8) according to Scheme 1.

Scheme 1



Example 1: Synthesis of 2-{(3R)-1-[2-(2,3-dihydro-1-benzofuran-5-yl)ethyl]piperidin-3-yl}-2,2-diphenylacetamide (1)

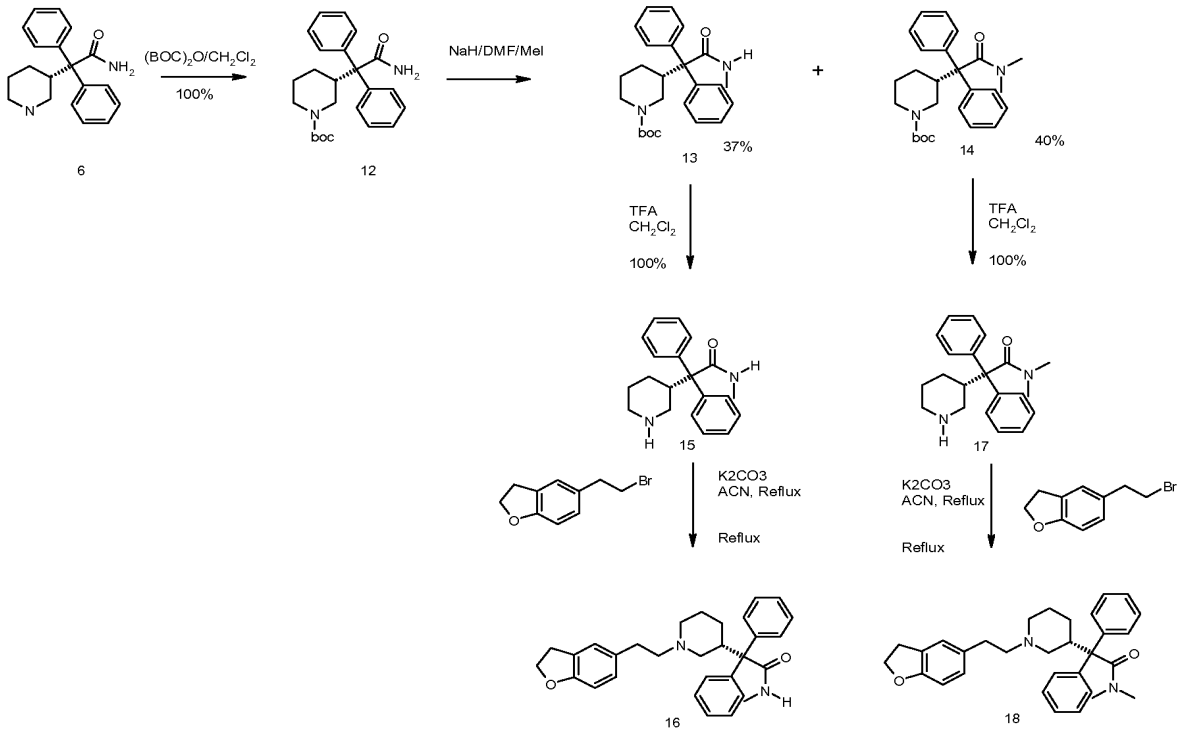
Synthesis of (1) was accomplished using the commercially available piperidine-3-ol (8) according to Scheme 1. The dl-mixture of piperidine-3-ol was resolved according to the method described in JCS Perkin Trans II (1981), 697 using D (+)-10-camphorsulfonic acid and 3R(+) hydroxypiperidine (+)-10-camphorsulfaonate (2) was obtained after recrystallization. (2) was then converted to 3-(1-carbamoyl-1,1-diphenylmethyl)piperidine (6) in four steps using the methods described in European Patent (EP 0 376 358 A1). (6) was

then allowed to react with (7) (prepared by a known procedure- Dunn et al, in J. Med Chem (1986) 2236) in the presence of potassium carbonate in acetonitrile at reflux to give (8). Compound (8) was used in the antiviral assay after in-house analysis by proton NMR and LCMS. 1H NMR (400 MHz, DMSO-d6) δ 7.30 (m, 10H), 7.03 (s, 1H), 6.98 (s, 1H), 6.86 (bs, 2H), 6.62 (d, *J* = 8Hz, 1H), 4.46 (t, *J* = 8Hz, 2H), 3.10 (t, *J* = 8Hz, 2H), 3.05 (bs, 1H), 2.81 (bs, 1H), 2.54 (bs, 2H), 2.32 (bs, 2H), 1.77 (m, 1H), 1.90-1.3 (m, 3H), 1.00 (bs, 1H), 0.50 (bs, 1H); LCMS (APCI+) for $C_{29}H_{32}N_2O_2$ *m/z* 441$(M + H)^+$.

Example 2: Synthesis of 2-{(3R)-1-[2-(2,3-dihydro-1-benzofuran-5-yl)ethyl]piperidin-3-yl}-N,N-dimethyl-2,2-diphenylacetamide (18)

The synthesis of (18) was achieved using amide (6) as starting material according to Scheme 2. Treatment of amide (6) with (BOC)2O in methylene chloride gave (12). Reaction of (12) with sodium hydride in dimethyl formamide followed by methyl iodide gave a mixture of the mono (13) and dimethylated (14) products which was then separated. Treatment of 14 with trifluoroacetic acid in dichloromethane gave free amine (17) which was then allowed to react with (7) in the presence of potassium carbonate in acetontirile in reflux to the title compound. Compound (18) was used in the antiviral assay after in-house analysis by proton NMR and LCMS. 1H NMR (400 MHz, DMSO-d6) δ 7.38 (m, 10H), 6.96 (s, 1H), 6.79 (d, *J* = 8Hz, 1H), 6.59 (d, *J* = 8Hz, 1H), 4.45 (t, *J* = 8Hz, 1H), 3.29 (s, 1H), 3.09 (t, *J* = 8Hz, 2H), 2.96 (bs, 1H), 2.80-2.30 (m, 4H), 2.42 (m, 1H), 2.20 (bs, 2H), 1.42 (bs, 2H), 0.91 (bs, 1H), 0.39 (bs, 1H); LCMS (APCI+) for $C_{31}H_{36}N_2O_2$ *m/z* 469 $(M + H)^+$.

Scheme 2



Compounds of the invention exhibit antiviral activity against HIV-1 in viral replication assays; however, the compounds are not active in HIV-1 cell-based assays that are dependent only on the early events in the HIV-1 replication cycle (e.g., HIV-1 entry, reverse transcription, and integration). Rather, the compounds act during a late event in the HIV-1 replication cycle to inhibit infectious virus production. More specifically, mechanism-of-action studies demonstrate that the compounds directly or indirectly inhibit HIV-1 gp160 processing during virion maturation, resulting in the production of virions with non-functional envelope proteins.

Cell Protection Assays

In cell protection assays, subject cells were infected with HIV-1 NL4-3, IIIB, NL4/IIIB, NL4/IIIBN-term, NL4/IIIBC-term, or NL4/IIIB5'Env at an moi of 0.08 or mock infected with medium only and added at 2 x 10^4 cells per well into 96 well plates containing half-log dilutions of test compounds. Six days later, 50 μl of XTT (1mg/ml XTT tetrazolium, 0.02 nM phenazine methosulfate) was added to the wells and the plate was reincubated for four hours. Viability, as determined by the amount of XTT formazan produced, was quantified spectrophotometrically by absorbance at 450 nm. Data from CPE assays were expressed as the percent of formazan produced in compound-treated cells compared to formazan produced in wells of uninfected, compound-free cells. The fifty percent effective concentration (EC_{50})

was calculated as the concentration of compound that effected an increase in the percentage of formazan production in infected, compound-treated cells to 50% of that produced by uninfected, compound-free cells. The 50% cytotoxicity concentration (CC_{50}) was calculated as the concentration of compound that decreased the percentage of formazan produced in uninfected, compound-treated cells to 50% of that produced in uninfected, compound-free cells. The therapeutic index (TI) was calculated by dividing the CC_{50} value by the EC_{50} value.

HIV-1 p24 assays

PM1 cells were infected with HIV-1 NL4-3 virus using an moi of 0.16 for 2 hours. Infected cultures were then washed with RPMI resuspended in 5 mls of RPMI medium at final cell densities of 2 x 10^5 cells/ml and added at 1 x 10^4 cells per well into 96 well plates containing half-log dilutions of test compounds. Five days after infection, virus replication was measured by quantifying HIV-1 p24 antigen present in the supernatants of infected cell cultures using the COULTER™ HIV-1 p24 antigen assay kit (Beckman Coulter, Miami, FL) according to the manufacturer's protocol. The fifty percent effective concentration (EC_{50}) was calculated as the concentration of compound that effected a decrease in p24 production in the supernatants of infected, compound-treated cells to 50% of that produced in the supernatants of uninfected, compound-free cells. The 50% cytotoxicity concentration (CC_{50}) was measured in PM1 cells using the XTT dye reduction method described above.

HIV co-culture assay

HIV co-culture (i.e., HIV Rep) assays were performed as described elsewhere (Cao, J., Isaacson, J., Patick A. K., and W. S. Blair. 2005. "A high throughput HIV-1 full replication assay that includes HIV-1 Vif as an antiviral target." *Antimicrob. Agents Chemother*. 49:3833-3841). Briefly, Half-log dilutions of test compounds were added to HeLa CD4/LTRBGal indicator cells seeded in 96-well plates at a cell density of 1 x 10^4 cells per well in DMEM or RPMI (Life Technologies) containing 10% FBS (HyClone). MT-2, CEM-SS, PM1, C8166, CEM4, or 174XCEM cells were infected with HIV-1 NL4-3 virus at an moi of 0.1-0.2. Two hours after infection, infected cells were washed with RPMI and resuspended in RPMI medium and then added to the 96-well plates containing compound-treated or compound free HeLa CD4/LTRBGal indicator cells. Virus replication was measured 4 days after infection by quantifying HIV-1 Tat induced □-Gal activity in the HeLa CD4/LTRBGal indicator cells using the Dual-Light™ System according to the manufacturer's protocol (Applied Biosystems). Data from the reporter gene measurements were expressed as the percent of reporter gene activity in infected compound-treated cells relative to that of infected, compound-free cells. The fifty percent effective concentration (EC_{50}) was calculated as the concentration of compound that effected a decrease in the percentage of the virally encoded reporter gene activity in infected, compound-treated cells to 50% of that produced in infected, compound-free cells.

Single-cycle infectious HIV-1 reporter viruses

HIV-1 single-cycle infectious reporter virus constructs based on HIV-1 NL4-3 were generated. To achieve this, the pNL4-3 plasmid was digested with the restriction endonucleases EcoRI and BamHI and a 2722 bp fragment containing HIV-1 NL4-3 Env sequences (NL4-3 nt 5743 to 8465) was isolated. The 2722 bp fragment was then ligated to pGEM4Z (Promega), which was digested with the same restriction enzymes (EcoRI and BamHI) to generate plasmid pNL4Env. The pNL4Env plasmid was then digested with BglII, separated from a 580 bp Env fragment (NL4-3 nt 7031 to 7611), and re-ligated to generate pNL4EnvΔBglII. A 372 bp fragment from the HIV-1 NL4-3 cDNA was amplified by PCR using oligonucleotide primers corresponding to NL4-3 sequences 8413 to 8448 (primer 1) and 8759 to 8785 (primer 2). Primer 2 encoded unique XbaI, NcoI, and XhoI endonuclease restriction sites 5' proximal to sequences corresponding to HIV NL4-3. The PCR amplified product was digested with the BamHI and XhoI endonucleases and ligated to the HIV-1 NL4-3 cDNA digested with the same enzymes to construct pNL4-ΔNef. A 705 bp fragment from the HIV-1 NL4-3 cDNA was then amplified by PCR using oligonucleotide primers corresponding to NL4-3 sequences 9038 to 9064 (primer 3) and 9714 to 9743 (primer 4). Primer 3 encoded a unique XhoI endonuclease restriction site 5' proximal to sequences corresponding to HIV NL4-3, while primer 4 encoded a unique NcoI restriction site 5' proximal to sequences corresponding to HIV NL4-3. The PCR amplified product was digested with the NcoI and XhoI endonucleases and ligated to pNL4-ΔNef digested with the same enzymes to construct pNL4-ΔNefΔNcoI. Firefly Luciferase sequences were amplified by PCR using primers that introduced unique XbaI and XhoI restriction endonuclease sites 5' and 3' of luciferase coding sequences, respectively. The luciferase PCR products were digested with the endonucleases XbaI and XhoI and ligated to pNL4-ΔNefΔNcoI digested with the same enzymes to construct pNL4-ΔNefΔNcoILuc. The plasmid pNL4EnvΔBglII was digested with the restriction endonucleases EcoRI and BamHI and a 2142 bp fragment encoding HIV-1 NL4-3 Env sequences containing a 580 bp deletion was isolated and ligated to pNL4-ΔNefΔNcoILuc digested with the same enzymes to generate pHIVΔEluc. To construct an HIV-1 Env expression vector, two oligonucleotides (5'-CATGGCTGAGTAACTAGAGGGCC-3' and 5'-CTCTAGTTACTCAGCCATGCATG-3') were annealed and ligated to pNL4-3 digested with SphI and ApaI, creating pNL4-3ΔGag. To generate the single-cycle infectious viruses VSV/HIVLuc and NL4/HIVLuc, pHIVΔELuc was co-transfected into HEK 293 cells with either a vesicular stomatitis virus (VSV) envelope expression vector (obtained from Stratagene) or pNL4-3ΔGag using LipofectAMINE Plus according to the manufacturer's protocol (Invitrogen Life Technologies). 48-72 hours after transfection, the single-cycle infectious HIV reporter viruses were harvested from the supernatants of transfected cells. Titers ($TCID_{50}$) of the resulting viral stocks were determined after infecting HeLa CD4/LTRBGal target cell lines with serial dilutions of the viral stocks (Johnson and Byrington, 1990) and measuring firefly

luciferase activity or induced Beta-Galactosidase activity 72 hours after infection using either a firefly reporter gene assay kit (Promega, Madison, WI) or a Beta-Galactosidase reporter gene assays kit (Applied Biosystems, Foster City, CA), respectively.

Single-Cycle Infection assays

Half-log dilutions of test compounds were added to HeLa CD4/LTRBGal or MT-2 target cells, seeded in 96-well plates at a cell density of 1 x 10^4 cells per well in DMEM (Life Technologies) containing 10% FBS (HyClone) or 1.6 x 10^4 cells per well in RPMI (Life Technologies) containing 10% FBS (HyClone). Compound-treated or compound-free target cells were then infected with VSV/HIVLuc or NL4/HIVLuc at an moi of 0.03. Seventy-two hours after infection of the HeLa CD4/LTRBGal cells, viral infection was monitored by measuring the induction of the Beta-Galactosidase reporter gene present in the HeLa CD4/LTRBGal target cells using the Dual-Light™ System according to the manufacturer's protocol (Applied Biosystems). Alternatively, seventy-two hours after infection of the MT-2 cells, viral infection was monitored by measuring the expression of the firefly luciferase reporter gene encoded by the virus using the Dual-Luciferase Reporter Assay System according to the manufacturer's protocol (Promega). Data from the reporter gene measurements were expressed as the percent of reporter gene activity in infected compound-treated cells relative to that of infected, compound-free cells. EC_{50} values were calculated as the concentration of compound that effected a decrease in the percentage of the virally encoded reporter gene activity in infected, compound-treated cells to 50% of that produced in infected, compound-free cells.

Virus production Assays

In the virus production assay, an envelope deleted NL4-3 reporter virus cDNA (pNL4-3ΔEnv) was co-transfected into HEK 293 cells with either a VSV-G expression vector, an HIV envelope expression vector, or both vectors using LipofectAMINE Plus according to the manufacturer's protocol (Invitrogen Life Technologies). Compound (compound I or NFV) was then added to transfected cell cultures at 2x EC_{90} concentrations (15 uM and 0.12 uM for Compound I and NFV, respectively) 3 hours after transfection. The supernatants of transfected cells were then harvested 72 hours after transfection and infectious virus production was measured by quantifying luciferase reporter gene activity after various dilutions of the supernatants of transfected cells were incubated in the presence of MT-2 cells for 72 hours. Results are presented as percent inhibition of the luciferase reporter signal in infected MT-2 cells in the presence of compound relative to that observed for the no compound control.

Construction of HIV-1 NL4/IIIB recombinants

Total DNA was isolated from HIV-1 IIIB infected cells and HIV-1 IIIB sequences corresponding to nucleotides 5729 to 8487 of HXB2 (accession number AF033819) were amplified by PCR using the oligonucleotide primers 5'-GGAAGCCATAATAAGAATTCTGCAACAACTGC-3' and 5'-

GTGCCAAGGATCCGTTCACTAATCGAATGG-3'. The PCR products were digested with the BamHI and EcoRI restriction endonucleases and ligated to pNL4-3 digested with the same restriction enzymes to generate the pNL4/IIIB chimeric cDNA, which contains IIIB sequences 5743 to 8480. To construct additional NL4/IIIB Env chimeras, pNL4/IIIB was digested with either EcoRI and NheI or BamHI and NheI. The N-terminal (EcoRI and NheI) or C-terminal (BamHI and NheI) IIIB Env containing fragments were isolated and ligated to pNL4-3 digested with either EcoRI and NheI or BamHI and NheI to generate the plasmids pNL4/IIIBN-term (containing IIIB sequences 5743 to 7264) and pNL4/IIIBC-term (containing IIIB sequences 7264 to 8480), respectively. Plasmid pNL4/IIIB5'Env was constructed after 3 different PCR reactions (see PCR reactions A, B and C below) using 2 plasimd templates (pNL4-3 and pNL4/IIIB) and 4 oligonucleotide primers: (1) 5'-AATAAGAATTCTGCAACAACTGCTGTTTA-3', (2) 5'-GAAGACAGTGGCAATGAGAGTGAAGGAGA-3', (3) 5'-CACTCTCATTGCCACTGTCTTCTGCTCTTTCT-3', and (4) and 5'-ATTGTTCTCTTAATTTGCTAGCTATCTGT-3'. PCR reaction A: Primers (1) and (3) were used to amplify a 491 bp fragment from pNL4-3 by PCR corresponding to pNL4-3 nucleotide sequences 5738 to 6229 (accession number AF324493). PCR reaction B: Primers (2) and (4) were used to amplify a 1069 bp fragment from pNL4/IIIB by PCR corresponding to HXB2 nucleotide sequences 6212 to 7281 (accession number AF033819). PCR reaction C: The two products from PCR reactions A and B were then combined and amplified by PCR using primers (1) and (4), yielding a 1560 bp product. The product of PCR reaction C was digested with EcoRI and NheI and ligated to pNL4/IIIB digested with the same restriction enzymes to generate plasmid pNL4/IIIB5'Env, which contains IIIB sequences 6225 to 7264. To generate virus, pNL4/IIIB, pNL4/IIIBN-term, pNL4/IIIBC-term, or pNL4/IIIB5'Env was transfected into HEK 293 cells using LipofectAMINE Plus according to the manufacturer's protocol (Invitrogen Life Technologies). 48-72 hours after transfection, infectious HIV-1 virus was harvested from the supernatants of transfected cells. Titers ($TCID_{50}$) of the resulting viral stocks were determined after infecting MT-2 T- cell lines with serial dilutions of the viral stocks (Johnson and Byrington, 1990) and monitoring cytopathic effect.

HIV Envelope Expression Assays

For the HIV Env expression assays, an HIV Tat/Env expression vector (pNL4-3ΔGag) was transfected into HEK 293 cells using LipofectAMINE Plus according to the manufacturer's protocol (Invitrogen Life Technologies), and the transfected cells were either treated with 2 x EC90 concentrations of compound (compound 1, NFV, or ATA) 3 hours after transfection or incubated in the absence of compound treatment. 24 hours after transfection, 2×10^4 transfected cells were co-cultured with 1×10^4 HelaCD4/LTRBGal target cells in a 96-well microtiter plate in the presence or absence of compound. Functional envelope expression was then measured by monitoring induction of Beta-Galactosidase expression in the target cells 48 hours after co-culture using the Dual-Light™ System according to the manufacturer's protocol (Applied Biosystems). Results are presented as percent inhibition of

the Beta-Galactosidase reporter signal in the presence of compound when compared to that observed for the no compound control.

HIV gp120 Western Analyses

For the Western analyses, HEK 293 cells were transfected with pNL4-3, pNL4/IIIB, or mock transfected and the transfected cells and supernatants of transfected cells were harvested 72 hours after transfection. Infectious virus production was measured using a portion of the supernatants of transfected cells in an HIV-1 co-culture as described above. In addition, the transfected cells were washed with cold PBS (Invitrogen, Carlsbad, California), and lysed with Western lysis buffer [50 mM Tris-Cl pH 7.2, 0.15 M Sodium Chloride (Sigma-Aldrich, St Louis, MO), 0.1% Sodium Dodecyl Sulfate (Sigma-Aldrich), and 1 % Triton-X 100 (Fisher Scientific, Fairlawn, New Jersey)]. The remaining supernatants of transfected cells were first clarified by centrifugation in a Sorval RC-7 (Kendro Laboratory Products, Newtown, CT) for 5 minutes at 1000 rpm (200 x g) at 4^0C. One ml aliquots were then layered on a 0.25 ml 20% sucrose (Sigma-Aldrich)/PBS cushion and centrifuged in an Eppendorf 5417C microcentrifuge (Brinkman Instruments, Westbury, NY) at 4^0C, 14,000 RPM (20,000 x g) for 3 hours to pellet the virions. The supernatant was removed and the virus pellet was resuspended in 0.050 ml Western lysis buffer. Proteins in each sample were separated on a 4-12% NuPAGE Bis-Tris gel using the MOPS buffer system (Invitrogen Life Technologies, Carlsbad, CA) and transferred onto PVDF membrane (Bio-Rad, Hercules, CA). Blots were probed either with a 1:1,000 dilution of a mouse monoclonal anti-HIV-1 p24 antibody (ICN Pharmaceuticals, Inc., Aurora, OH) or a 1:2500 dilution of rabbit polyclonal antiserum directed against HIV-1 gp120 (Advanced Biotechnologies, Columbia, MD). Proteins were subsequently detected using the Tropix Western-Star chemiluminescence kit (Applied Biosystems, Foster City, CA) and visualized by exposure to Kodak BioMax MR-1 film (Kodak Scientific Imaging Systems, New Haven, CT). The Western analyses were quantified by densitometer scanning using an Alpha Innotech Chemilmager (Alpha Innotech Corporation, San Leandro, CA) and the 1D Multi-densitometry software provided by the manufacturer.

HIV-1 antiviral screen and mechanism-of-action

In cell protection assays using the HIV-1 NL4-3 strain and MT-2 T-cell lines, compound 1 exhibited a 50% efficacious concentration (EC_{50}) of 0.82 uM (Table 1) and 50% cytotoxic concentration (CC_{50}) of 53 uM, resulting in a therapeutic index (TI) of 65. Similarly, the compound showed an EC_{50} value of 2.6 uM and CC_{50} value of 60 uM in antiviral assays using HIV-1 NL4-3, PM-1 cells and a p24 endpoint (Table 1). The antiviral activity of compound 1 was cell line independent. As shown in Table 2, compound 1 exhibited similar EC_{50} values against HIV-1 NL4-3 in antiviral assays using MT-2, CEM-SS, PM1, C8166, CEM4, or 174XCEM cell lines. Alternatively, compound 1 exhibited reduced activity in antiviral assays using the HIV-1 IIIB, or HIV-1 Ba-L virus strains and either MT-2, or PM1 T-cell lines, respectively. EC_{50} values of >32 and 11 uM were measured for compound 1 against the HIV-1 IIIB, and Ba-L strains, respectively (Table 1). These data demonstrate that

compound 1 exhibits antiviral activity against at least one laboratory HIV-1 strain (NL4-3) and suggest that the compound targets a less conserved segment of the viral genome.

As part of an initial effort to characterize the target of compound 1, the compound was evaluated in a panel of HIV-1 infection assays, which utilize single-cycle infectious HIV reporter viruses packaged with either an HIV envelope (NL4-3) or an envelope derived from the vesicular stomatitis virus (VSV) (Figure 1). In such assays, target cells are infected in the presence or absence of compound with either the HIV-1 or VSV enveloped reporter viruses and infection is measured by monitoring the expression of either a viral encoded reporter gene or a reporter gene present in the target cell that is induced after viral infection. In all cases, reporter gene expression in the single-cycle infection assay is dependent on viral entry, reverse transcription, integration and Tat-mediated HIV-1 gene expression, but not the late stages of the viral replication cycle (e.g., virion maturation or particle production). Therefore, based on their corresponding inhibition profiles in single-cycle infection assays, HIV compounds can be categorized as entry, early, or putative late-stage inhibitors of HIV-1 replication. For example, HIV-1 specific entry inhibitors (e.g., gp120 or CXCR4 inhibitors) would be expected to inhibit infection by the HIV-1 enveloped reporter virus but not the VSV-G enveloped reporter virus. Alternatively, early stage inhibitors (e.g., RTIs or INIs) would be expected to inhibit infection by both viruses, while late stage inhibitors (e.g., PIs) would not be expected to inhibit infection by either virus as such late stage events are not measured in the assay. This is illustrated with the control compounds included in the assay (ATA, NFV, and EFV). ATA (the HIV-1 entry inhibitor control) inhibited infection of HeLa CD4/LTRBGal cells by the HIV enveloped reporter virus (NL4/HIVLuc) with an EC_{50} value of 0.069 uM (Table 3). However, ATA did not inhibit infection by the VSV-G enveloped reporter virus (VSV/HIVLuc). Alternatively, EFV (the NNRTI control) inhibited infection of HeLa CD4/LTRBGal cells or MT-2 cells by both reporter viruses with EC_{50} values of 1-2 nM, while NFV (the PI control) was not active against either of the single-cycle viruses (Table 3). The profile of compound 1 was most similar to that of NFV (i.e., not active against either single-cycle reporter virus), thus suggesting that compound 1 may target a late event in the HIV-1 replication cycle (Table 3).

To confirm that compound 1 targets a late event in the HIV-1 replication cycle, the compound was evaluated in virus production assays. In the virus production assay, an envelope deleted NL4-3 reporter virus cDNA was co-transfected into HEK 93 cells with either an HIV envelope expression vector, a VSV-G expression vector, or both vectors (Fig. 1). Compound (compound 1, or NFV) was then added to transfected cell cultures at 2x EC_{90} concentrations 3 hours after the transfection. The supernatants of transfected cells were then harvested 72 hours after transfection and infectious virus production was quantified as described in Materials and Methods. The results showed that compound 1 inhibited the production of infectious viruses packaged with an HIV-1 envelope (99% inhibition; Table 4); however, the compound did not inhibit the production of infectious HIV-1 virions packaged with a heterologous envelope (VSV-G). In fact, co-expression of VSV-G with the HIV-1 Env

(NL4-3) rescued the production of infectious virus from transfected cells in the presence of compound 1 (Table 4). As expected, the PI control (NFV) inhibited virus production (84 – 97%) in an envelope-independent manner. These data demonstrate that compound 1 inhibits virus production in an envelope-dependent manner. Furthermore, the results suggest that compound 1 may target the HIV-1 envelope during virion maturation or production.

Results from the virus production experiments suggest that compound 1 targets the HIV-1 envelope during virion maturation, resulting in the production of non-infectious virions. To provide evidence supporting this hypothesis, we utilized our HIV-1 IIIB virus stock, which exhibited a reduction in susceptibility to compound 1 when compared to NL4-3 (Table 1). A recombinant NL4/IIIB virus was constructed containing sequences derived from HIV-1 IIIB (nucleotides 5743 to 8475), which includes the complete coding regions for gp120, Vpu and Tat as well as portions of the Vpr, Rev, and gp41 coding regions. The activity of compound 1 was evaluated in susceptibility assays against the NL4/IIIB recombinant as well as the parental NL4-3 virus as described in Experimental Procedures. The results showed that the NL4/IIIB recombinant virus exhibited a >27-fold reduction in susceptibility to compound 1 when compared to NL4-3 (Table 5). These results are similar to that observed for the HIV-1 IIIB virus (Table 1) and thus demonstrate that HIV-1 IIIB sequences corresponding to nucleotides 5743 to 8475 contain critical determinants of compound 1 susceptibility.

As part of an initial effort to more precisely define the IIIB Env sequences that confer resistance to compound 1, 3 additional NL4/IIIB chimeric viruses were constructed. The first chimera (NL4/IIIBN-term) contains IIIB sequences corresponding to nucleotides 5743 to 7264, which includes the N-terminal half of the gp120 coding region, the complete Vpu coding region, and partial coding regions for Vpr and Tat. The second chimera (NL4/IIIBC-term) contains IIIB sequences corresponding to nucleotides 7264 to 8480, which includes the C-terminal half of the gp120 coding region and partial coding regions for Tat, Rev, and gp41. The third chimera (NL4/IIIB5'Env) contains IIIB sequences corresponding to nucleotides 6225 to 7264, which includes the complete gp120 coding region as well as portions of the Vpu, Tat, Rev, and gp41 coding regions. The activity of compound 1 was evaluated in susceptibility assays against each of the chimeric viruses as well as the parental NL4-3 virus as described in Experimental Procedures. The results showed that compound 1 exhibited antiviral activity against NL4/IIIBN-term with an EC_{50} value similar to that observed for the NL4-3 parent (Table 5). In addition, compound 1 exhibited a modest 4-fold reduction in activity against NL4/IIIBC-term when compared to NL4-3 (Table 5). Alternatively, the NL4/IIIB5'Env chimeric virus exhibited a >27-fold reduction in susceptibility to compound I when compared to NL4-3 (Table 5). The susceptibility results for NL4/IIIB5'Env are similar to those observed for the NL4/IIIB recombinant and HIV-1 IIIB viruses (Fig 2.; Table 1) and demonstrate that HIV-1 IIIB sequences corresponding to nucleotides 6225 to 7264 are sufficient to recapitulate the resistant phenotype observed for the HIV-1 IIIB virus. Therefore, the data in Table 5 strongly

suggest that HIV-1 Env is the target of compound 1. Furthermore, these data suggest that multiple IIIB Env sequences may be required to confer compound 1 resistance.

To confirm compound 1 targets HIV-1 Env function, an HIV Env expression assay was utilized (Fig. 2). In such an assay, an HIV Tat/Env expression vector (pNL4Env) was transfected into HEK 293 cells and transfected cells were either treated with compound (compound 1, NFV, or ATA at their respective 2x EC90 concentrations) 3 hours after transfection or incubated in the absence of compound treatment. 24 hours after transfection, the transfected cells were co-cultured with HelaCD4/LTRBGal target cells in the presence or absence of compound. Functional envelope expression was then measured by monitoring induction of Beta-Galactosidase expression in the target cells, which is dependent on HIV Env-mediated cell fusion and Tat transfer to the target cells. The results showed that compound 1 inhibited the expression of a functional HIV NL4-3 Env (84% inhibition) if present during the both the transfection step and the co-culture step of the assay (I & II; Table 6). However, compound 1 did not significantly inhibit HIV NL4-3 Env function if the compound was only present during either the transfection step (I) or the co-culture step (II). HIV Env expression occurs throughout the assay and the absence of compound during either the transfection or the co-culture step allows enough functional Env expression to mediate cell fusion. In addition, compound 1 did not inhibit the expression of a functional HIV-1 IIIB Env (Table 6). This latter result is consistent with the antiviral assays presented above, which showed that viruses encoding the HIV-1 IIIB Env were not susceptible to inhibition by compound 1 (Table 1 and Table 5). Alternatively, the HIV entry inhibitor control (ATA) inhibited HIV-1 NL4-3 and IIIB Env function if the compound was present during the co-culture step (II or I & II), while NFV did not significantly inhibit HIV Env function (Table 6). These results demonstrate that compound 1 inhibits the expression of a functional HIV-1 NL4-3 envelope and confirm that the compound does not target HIV Env-mediated fusion.

To determine if HIV Env protein expression was directly inhibited by compound 1, we performed Western analyses using a gp120 specific polyclonal antibody (Ab) (Fig. 3). In the experiment shown in Fig. 3, cells were transfected with the HIV-1 NL4-3 infectious cDNA or an HIV-1 NL4/IIIB chimeric cDNA in the presence of various concentrations of compound 1 or the absence of compound. 48 hours after transfection, cells were harvested and extracts of the harvested cells used in the Western analysis. In addition, infectious virus production was measured in the supernatants of transfected cells as described in Materials and Methods. Consistent with that expected, concentrations of compound 1 corresponding to the 0.5, 1.0, 7.5, and 15 uM inhibited NL4 infectious virus production by 12, 44, 99, or 100%, respectively (Fig. 3A). Alternatively, NL4/IIIB infectious virus production was not affected by compound 1 at concentrations of 15 uM, which corresponds to the 2x EC_{90} value determined in antiviral assays. In the Western analysis, two distinct protein molecular weights were detected by the gp120 Ab, which were not present in the no virus control. The protein molecular weights detected were consistent with that expected for gp120 and gp160. Quantification of the

Western analysis showed that gp160 and gp120 were present in an approximate 1:1 ratio in the absence of compound in transfected cells. In the presence of compound, a concentration-dependent increase in the gp160 precursor was observed. In fact, compound 1 at concentrations of 15 uM (i.e., 2x EC_{90} concentrations) gp160 represents 76% of the total envelope protein present in transfected cells. Alternatively, the NL4/IIIB gp160/gp120 ratio was not altered in the presence of 2x EC_{90} concentrations of compound 1. This is consistent with the observation that NL4/IIIB chimeric virus replication is not inhibited by compound 1 (Table 5). A separate Western analysis was performed on the same samples using a p24 specific Ab. The data showed that HIV-1 Gag processing was not affected by compound I in cells transfected by either HIV-1 NL4-3 or NL4/IIIB (Fig. 3B). These data strongly suggest that compound 1 specifically interferes with gp160 processing, resulting in a defect in HIV infectivity.

To determine if compound 1 affects the incorporation of HIV-1 Env proteins into virions, a Western analysis was performed using a gp120 Ab and isolated virions produced in the presence or absence of 2x EC_{90} concentrations of compound 1 or NFV (Fig. 4). As with the previous experiment, infectious virus production was measured in the supernatants of transfected cells as well. The results showed that 2x EC_{90} concentrations of compound 1 and NFV inhibited infectious virus production by 100 and 94%, respectively (Fig. 4). The Western analyses showed that the majority of the HIV-1 Env protein present in virions in the absence of compound or in the presence of NFV is in the form of gp120 (76 and 80%, respectively) (Fig 4). These data are consistent with previous reports (Dubay et al., 1995) and show that although both gp160 and gp120 are present in transfected cells (Fig 3), gp120 is incorporated into virions more efficiently (Fig 4). Alternatively, virions produced in the presence of 2x EC_{90} concentrations of compound 1 contain almost exclusively unprocessed gp160 Env protein (Fig 4). In addition, the relative quantities of total HIV-1 Env protein (i.e., gp160) appear to be ~50% reduced in the compound treated virions when compared to untreated virions. These data demonstrate that compound 1 directly or indirectly inhibits gp160 processing resulting in the incorporation of non-functional (i.e., unprocessed) Env proteins in HIV virions.

Compounds 1 to 14 were evaluated in cell protection assays using the HIV-1 NL4-3 strain and MT-2 T-cell lines. The antiviral activities of compounds are presented in terms of their 50% effective concentration values (EC_{50}) and compound cytotoxicity measurements are presented as 50% cytotoxic concentration (CC_{50}) values. In addition, a therapeutic index (TI) is calculated for each compound by dividing the CC_{50} value by the EC_{50} value. Antiviral activity is defined here as a TI $\geq$ 5, and, as shown in Table 7, all 14 compounds exhibit antiviral activity.

Table 1 presents the antiviral activity of compound 1 against different HIV-1 laboratory strains. For the P24 assay, Antiviral activity was determined after measuring p24 production six days after infection of PM1 cells with HIV-1 NL4-3 or HIV-1 Ba-L. Cytotoxicity

was determined by measuring cell viability using the XTT dye reduction method six days after compound addition to PM1 cells.

Table 2 presents the antiviral activity of compound 1 in different T-cell lines.

Table 3 presents the activity of compound 1 in HIV-1 single–cycle infection assays.

Table 4 presents the activity of compound 1 in HIV-1 virus production assays. HIV-1 infectious virus production measured after co-transfection of either an HIV-1 envelope expression vector (NL4-3) or a VSV envelope expression vector (VSV-G) and an HIV-1 reporter virus cDNA containing a mutation in envelope sequences. Results presented as percent inhibition of infectious virus production relative to the no compound control.

Table 5 data show that the HIV-1 envelope sequences were sufficient to confer susceptibility to compound 1. The fold-change was determined by dividing the EC50 value obtained for each IIIB recombinant by the EC50 value observed for the NL4-3 parental virus.

Table 6 data show that compound 1 inhibited the expression of a functional HIV envelope. These data represent the percent inhibition of NL4-3 or NL4/IIIB functional envelope expression in the presence of compound added during either the transfection step (I), during the co-culture step (II) or during both steps (I & II) relative to the no compound control (Refer to Fig. 2).

Table 7 presents the antiviral activity of compounds 1 to 14 in the cell protection assays.

Table 1

			Antiviral Activity		
Virus	Cell Line	Assay	EC50 (uM)	CC50 (uM)	TI
HIV-1 NL4-3	MT-2	XTT	0.82	53	65
HIV-1 NL4-3	PM1	P24	2.6	60	23
HIV-1 IIIB	MT-2	XTT	>32	>32	ND
HIV-1 Ba-L	PM1	P24	11	60	5

Table 2

	EC50 (uM)		
Cell Line	Compound 1	EFV	NFV
MT-2	0.78	0.001	0.022
CEM-SS	1.1	0.0003	0.011
PM1	0.93	0.0009	0.013
C8166	0.69	0.001	0.047
CEM4	0.95	0.0007	0.012
174XCEM	0.79	0.0008	0.014

Table 3

		EC50 (uM)			
Virus	Target Cell	Compd 1	EFV	ATA	NFV
VSV/HIVLuc	HeLa	>32	0.001	>76	>1
VSV/HIVLuc	MT-2	>10	0.001	ND	ND
NL4/HIVLuc	HeLa	>32	0.002	0.069	>1
NL4/HIVLuc	MT-2	>10	0.002	ND	ND

Table 4

Plasmids Transf.		% Inhibition	
Env	Reporter	Compound 1	NFV
NL4-3	HIVLuc	99	88
VSV-G	HIVLuc	<1	97
NL4-3 + VSV-G	HIVLuc	<1	84

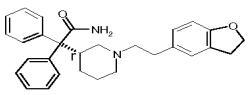
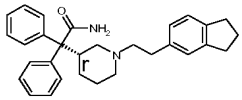
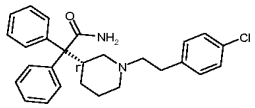
Table 5

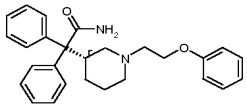
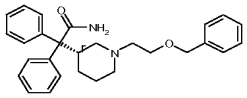
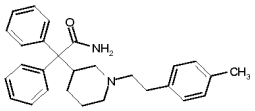
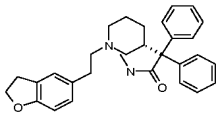
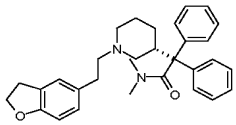
Virus	EC50 (uM)	Fold-Change
NL4-3	1.2	-
NL4/IIIB	>32	>27
NL4/IIIBN-term	1.2	1
NL4/IIIBC-term	5.3	4
NL4/IIIB5'Env	>32	>27

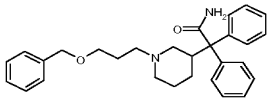
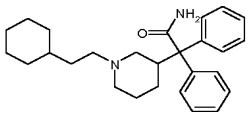
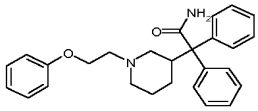
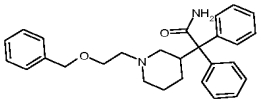
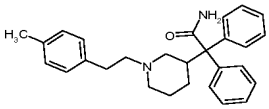
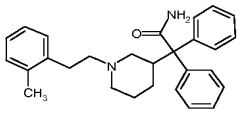
Table 6

Compound	NL4-3 (%Inhib.)			NL4/IIIB (%Inhib.)		
	I	II	I & II	I	II	I & II
Compound 1	<1	14	84	2	<1	<1
ATA	6	99	99	11	99	99
NFV	6	<1	<1	ND	ND	ND

Table 7

		Antiviral Activity		
Compound	**Structure**	**EC50 (uM)**	**CC50 (uM)**	**TI**
1		0.82	53	65
2		0.12	6	49
3		0.55	19	35

4		1.1	31	29
5		0.45	18	41
6		2.0	39	19
7		5.9	156	26
8		3.8	47	12

9		3.9	51	13
10		4.7	40	9
11		5.4	54	10
12		5.6	51	9
13		1.0	53	52
14		1.4	52	36

The examples and preparations provided above further illustrate and exemplify the compounds of the present invention and methods of preparing such compounds. It is to be understood that the scope of the present invention is not limited in any way by the scope of these examples and preparations.